PROTEIN TURNOVER IN MICE SELECTED FOR APPETITE.

Catherine A. L. Parker, BSc (hons) Nottingham

Thesis submitted to the University of Edinburgh for the degree of Doctor of Philosophy.

Metabolic Fitness Group,
Department of Molecular and Physiological Genetics,
Agricultural and Food Research Council,
Institute of Animal Physiology and Genetics Research,
Roslin,
Midlothian,
EH25 9PS.

May 1988

Declaration

I hereby declare that this thesis has been composed by myself and has not been submitted for any other degree in Edinburgh or elsewhere. The work presented herein is my own and all work of other authors is duly acknowledged. I also acknowledge all assistance given to me during the preparation of this thesis.

Catherine A. L. Parker

ACKNOWLEDGEMENTS

I am very grateful to my supervisors, Drs. J.A. O'Brien (Department of Biochemistry) and P.A. Sinnett-Smith (Institute of Animal Physiology and Genetics Research, IAPGR) for their guidance, support and encouragement throughout this period of study. Many thanks also to the late Dr. R.B. Land (formerly Deputy Director IAPGR) for providing necessary facilities to perform this work.

I am obliged to Professor W.G. Hill (Department of Genetics) for providing the appetite selected mice. I am also indebited to all the staff of the Genetics mouse house involved in the day to day care of the mice and also to Mr. I. Hastings (Department of Genetics) and Mr. S.J. King (formerly Department of Genetics) who were concerned with the extremely time consuming job of mouse selection and breeding. I am grateful to Miss P.J. Cook (formerly Department of Genetics) for supplying some mouse plasma samples. Mr. D. Parnham (IAPGR) is also thanked for providing mice.

Life in the laboratory was infinitely enhanced by Miss A. Chong (IAPGR), Dr. L.M. Harrison (IAPGR), Mr. A.R.D. MacLeod (IAPGR), Mr. J. Ritchie (North East London Polytechnic; NELP), Mr. P. Sharma (NELP), Mr. M. Sohodele (NELP) and last but certainly not least Mr. P. Wilson (IAPGR). Special thanks must go to Miss A. Chong for performing the time course study. The invaluable help of Dr. L.M. Harrison, Mr. J. Ritchie, Mr. P. Sharma, Dr. P.A. Sinnett-Smith, Mr. M. Sohodele and Mr. P. Wilson at various times during mouse dissection is gratefully acknowledged. Finally, in connection with the laboratory is 'Mrs. Mac.' (Mrs. B. McKenzie, IAPGR) who works wonders with dirty glassware and deserves a special mention for this and for adding her own brand of fire and brimstone to Dryden Laboratory.

Many people have been willing to impart knowledge and expertise in their specialist fields. I am obliged to Dr. M. Mitchell (Institute of Grassland and Animal Production, IGAP) for discussion on thyroid hormone assays and thyroidectomizing agents and to Mr. D. Waddington (IAPGR) for statistical advice. Particular thanks go to Mr. J.A. Woolliams (IAPGR) who has always been ready to give helpful criticism, discussion and advice at a moments notice. The assistance of all library staff at IAPGR is acknowledged (Mrs. A. Barfield, Mrs. I Grieg, Mrs. R. MacDonald and Miss. D. Meikle) as well as secretarial help from Mrs. J. Cherrie (IAPGR) and Mrs. J. Smith (IGAP).

Finally, I wish to thank my family for their infinite support as always.

Read not to contradict nor to believe but to weigh and consider.

Bacon

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Selection for more efficient meat producing animals has met with limited success; the aim of these studies was to investigate metabolic differences between divergently selected strains of mice to attempt to discover metabolic characters that may provide better selection traits than traditional selection criteria such as postweaning gain.

The mice studied were divergently selected for high and low food intake which resulted in high appetite mice being heavier and slightly leaner although with higher maintenance requirements than the low line.

The fractional rates of protein synthesis and degradation were determined in the gastrocnemius muscle, liver and small intestine. It was found that selection for low appetite significantly increased the rate of skeletal muscle protein turnover (H, C, L; 15.2, 15.0, 18.2%/d) but had little effect upon this parameter in liver and small intestine. This first study suggested therefore, that skeletal muscle protein turnover was more efficient and maintenance energy requirements less in high than low appetite mice. This contrasts with maintenance requirements for the whole body.

Since thyroid hormones have been associated with both appetite and protein turnover regulation, this group of hormones was investigated to see if they were involved in this variation in protein metabolism. Plasma total thyroxine concentrations were sigificantly lower in high than low appetite mice at five (H vs L, 44.9 vs 58.0

ng/ml) but not at six weeks of age. There was no variation in total tri-iodothyronine levels. These results were tenable with thyroid hormones affecting skeletal muscle protein turnover but not with a regulatory action upon appetite.

To determine if the variation in thyroid hormone concentrations observed above was concerned with regulation of muscle protein turnover, groups from both high and low lines were chemically thyroidectomized and half from each group were given a replacement dose of thyroxine at a set level. It was found that variation in the rate of protein turnover in skeletal muscle was unaffected by thyroid hormones but there was an indication that liver protein turnover in the high line was more responsive to thyroid hormones. This difference in response to thyroid hormones was not manifest in intact mice due to lower circulating thyroid hormone levels in the more responsive high line. Overall, thyroid hormones would have only a negligible effect upon whole body protein turnover despite their effect on liver.

Collectively, these results suggest that protein turnover is more efficient in high appetite selected mice than in low line counterparts although another aspect of metabolism must have much greater maintenance energy requirements in the high line in order to reverse this measurement for the whole body. Thyroid hormones were not important in controlling the variation in protein turnover in skeletal muscle nor in determining the level of food intake. However, they may be involved in another aspect of metabolism since concentrations differed between lines.

INTRODUCTION

The improvement of livestock production by selecting genetically meritricious individuals has traditionally been based on gross body measurements such as weight gain over a specified period or to a certain age. However, this type of selection is inefficient and slow. The phenotypic characteristics are the result of many individual biochemical processes. It is therefore difficult to relate a favourable genotype with phenotype. It has been argued that identifying animals of superior genetic merit could be improved by selecting individuals on biochemical parameters which are more directly related to an animal's genotype (Land, 1981). There are several problems concerning this type of selection criterion. Firstly, suitable biochemical markers must be found which have a considerable correlation with production. This is a major difficulty since important phenotypic characteristics are polygenic. Secondly, such markers must be simple, quick and cheap to measure to be commercially useful. Finally, the quality of the product must not be impaired but improved if possible.

Protein turnover processes are one of the largest drains on the metabolizable energy intake of an animal (section 1.2.3). In the growing animal the partitioning of metabolizable energy between maintenance and growth is very important in determining the energy available for weight gain. The maintenance energy requirements depend largely upon whole body rates of protein turnover. The most important aspect of growth in a meat animal is protein deposition. Therefore, protein metabolism is involved in two aspects of animal growth:

i) how much metabolizable energy is effectively "lost" from growth due to continuous protein turnover occurring with no net protein growth and

ii) how much of the metabolizable energy remaining after maintenance goes towards protein accretion as opposed to other anabolic processes such as fat deposition and bone growth.

Thus, characteristics of protein turnover and their control may be a reasonable area of metabolism in which a suitable selection marker may be sought.

There are three main ways of studying genetic differences. The first is to examine metabolic variation in different strains of a species with a wide phenotypic variation in a parameter such as body weight. However, this procedure is limited because strains are unrelated having random associations of many metabolic characteristics. The second is to use animals which have a major gene difference causing wide phenotypic variation between littermates. This solves the problem of having a closely related genetic base although the drawback is that such a major genetic difference is only rarely encountered in real production systems where normally a wide range of continuous variation occurs throughout the population. The other method involves divergently selecting animals from a common base population over a number of generations. This results in groups of animals with widely different phenotypes due to small changes at many gene loci yet their origin is the same. This approach is similar to the commercial situation and was adopted in this study.

The animals used were mice selected for divergence in food intake. The selection criterion was 4-6 week food intake with a correction by phenotypic regression for 4 week body weight. There were three contemporary lines, one selected for high appetite (H), one for low appetite (L) and an unselected control (C). These lines were replicated three times (replicates 1, 2 and 3). Selection was relaxed at generation 23 due to inbreeding and health problems. At this stage replicate 6 was formed by combining replicates 1, 2 and 3 and selection continued. These animals are described in detail in section 2.2 and by Sharp, Hill and Robertson (1984). Previous studies have shown that the high intake mice were larger yet leaner than their low intake counterparts. High line mice have higher maintenance requirements and are no more efficient than the L appetite animals (Bishop and Hill, 1985).

Despite mice being very different from sheep or a beef cow there are good reasons for using them as a model in this type of study. The usual arguments that using a laboratory species is more economic and much more information can be gathered in a shorter period are obvious. However, it may be reasoned that because mice are so much smaller than farm animals, many crucial measurements such as efficiency and requirements for maintenance energy will be vastly different and therefore irrelevant. This suspicion is unfounded since Taylor (1980a) established two genetic size-scaling rules that allow experimental results in one breed or species to provide a quantitative guide to biological relationships in other species. In essence the first rule says that all age and time variables are directly proportional to $A^{0.27}$ where A is mature weight and all cumulated inputs and outputs are directly proportional to A. The second rule scales cumulated variables to the standardized variable

(eg standardized food intake = FI/A). Both milk production and body growth from embryo to adult have been examined by this approach. Interspecies predictions were very consistent with actual results (Taylor, 1973, Taylor, 1980b).

The approach that is outlined above was adopted following a survey of conventional selection experiments in the literature as well as those in which protein metabolism in particular has been studied. Reviews of the conclusions of these surveys are given in sections 1.1 and 1.2. An additional requirement of biochemical selection markers is that for practical application they should be quick, simple and cheap to measure. It was therefore attempted to relate protein turnover processes to a more easily measureable yet still physiologically related character. Thyroid hormones were examined in this context and are reviewed in section 1.3. The reasons for examining these hormones in particular are discussed later (section 4.1).

CHAPTER ONE

LITERATURE REVIEW

1.1 THE GENETIC DIFFERENCES IN BODY GROWTH

It is necessary to establish the effects of conventional selection experiments upon parameters such as body growth and body composition in order that a biochemical approach to selection can be compared with the traditional method.

1.1.1 THEORY OF GENETIC SELECTION

The response to selection per generation depends upon the intensity of selection and the heritability of the selected character. Selection intensity is dependent upon the proportion of population in the selected group; the smaller this proportion the greater the response. Heritability is determined by the frequencies of genes segregating in a particular population and the size of their effects. Variation in realized heritabilities is caused by differences in gene frequencies in the base population and by drift during selection (McCarthy, 1982). Thus, the greater the heritability the greater the selection response.

To ensure that differences arising between control and selected animals are due to the selection process itself and are not the result of genetic drift, it is common policy to select several lines independently for the same trait. If similar correlations are obtained in all lines then one can attribute any differences to the selection process.

Several types of selection programme are commonly employed. Firstly, there is individual or mass selection. Individuals with the highest phenotypic values of the whole population are chosen. Secondly, there is family selection where whole families are selected as complete units according to their mean phenotypic value and any within family deviations are ignored. This method is chiefly used when the selected character has a low heritability. A variation on family selection is sibling selection. Sibling selection is performed when it is not possible to measure the character in the individuals to be parents of the next generation. For example, if an individual dies from a given disease then others from the same family are avoided even though they have not died. Therefore, the character is measured in as many siblings as possible and from these a mean family value is obtained. However, the selected individual does not contribute towards this value. Next, there is within family selection which is appropriate when a large component of environmental variation is common to members of a family rather than to the whole population. This situation applies to preweaning growth in pigs and mice where there is a very large maternal influence upon the young. Individuals with the largest deviation from the family mean are chosen regardless of the value of the family mean. This type of programme was used to select mice in this study. Hence, within family selection can be regarded as the converse of family selection since selection within the family gives all the weight to within family deviations and a zero weighting to family means whereas in family selection all the weight is given to family means and zero weighting to within family deviations. Finally, comes progeny testing which is used when the character cannot be measured in the parent and so a score is based upon

progeny performances. For instance, milk production capabilities cannot be directly measured in bulls. In this system the milk yield and quality of a given number of daughters are valued over the first two lactations. Upon the collective performance of the daughters a score is given to the bull. This type of testing is obviously very time consuming and expensive.

1.1.2 SELECTION FOR BODY GROWTH

1.1.2.1 Body Weight and Growth Rate

It was first necessary to ascertain that growth rate and body size can be altered by selection. The first experiments where animals were selected for body size established:

i) that body weight is genetically controlled since selection will change the mean body weight of the selected population in accordance with the direction of selection and

ii) that much of the variation in body weight has an additive genetic base (Goodale, 1938; MacArthur, 1944, 1949; Falconer, 1953).

Falconer (1953) also noted that progress was more rapid in the downwards direction. He attributed the difference in response to inbreeding depression. Many other studies have also selected mice for weight gain between certain ages as well as for body weight at a certain age (Rahnefield, Boylan, Comstock and Singh, 1963; Baker and Chapman, 1975; Barria and Bradford, 1981). There is a wide variation in the estimates of heritability between these experiments. Rahnefield et al (1963) obtained a heritability estimate of 0.18 for 3-6 week weight gain whereas Hanrahan, Eisen

and Legates (1973a) found a heritability of 0.35 for the same selection criterion. The estimates of heritability of mice selected for weight at a fixed age ranged from as small as 0.13 to as large as 0.55 (Cheung and Parker, 1974; Eisen, 1978).

Changes in body weight occur for many generations but eventually the selection limit is reached (Wilson, Goodale, Kyle and Godfrey, 1971). The generation at which this limit is obtained varies but a response usually continues past the 20th generation. In a long term study of more than 80 generations the selection limit for body weight was reached at generation 35 with further progress being made between generations 65 to 73 (Wilson et al, 1971). Barria and Bradford (1981) concluded that when the limit of selection was reached in their study it was due to a negative correlation between gain and fitness rather than exhaustion of additive genetic variance. A theory that further progress may be obtained after relaxation of selection was proposed (Lerner, 1954) since the rapid change in the relationship between the organism and the environment may be accompanied by a loss of plasticity in the organism. However, relaxation of pressure would allow plasticity to return. In conclusion, these early experiments established that body weight and related measurements were genetically controlled and so could be manipulated by selection.

1.1.2.2 Genetic Manipulation of Growth Curves

Being able to alter body size genetically is crucial but the rate of growth is also important. Obviously of two animals with the same final size, the one that reaches this size quicker is more efficient

provided of course the body composition remains suitable. This aspect is discussed in section 1.1.3. The use of growth curves is very useful in assessing how fast an animal grows.

A growth curve is a plot of body size against age and usually follows a sigmoidal pattern (Figure 1.1.2.2.1). Body size can be measured by parameters such as height and girth but weight is the most commonly used measure. This expression of body growth was first described by Brody (1945) and it is an invaluable biological model for understanding the overall growth of the body and its components although it gives no information on body composition. Brody (1945) distinguished the growth curve into two parts: the self accelerating phase (SAP) followed by the self inhibiting phase (SIP). During these periods the growth rate increases and decreases respectively (Figure 1.1.2.2.1). The transition between these two phases is the inflection point. The distinction between these periods is important because growth in SAP depends upon the gain yet to be achieved and hence potential growth can be altered. However, the potential growth during SIP is limited by the body weight that the animal has already achieved and so can only be manipulated to a lesser extent. The terms used to describe the shape of the growth curve are asymptotic weight (mature weight, A) and the relative rate at which maturity is reached ie the slope of the curve.

There can be several approaches to altering growth curve parameters. One strategy would be to select for animals with a greater mature weight. However, penalties would arise from this approach because animals with higher asymptotic weights take longer to reach maturity (Eisen, Lang and Legates, 1969; Fitzhugh, 1976;

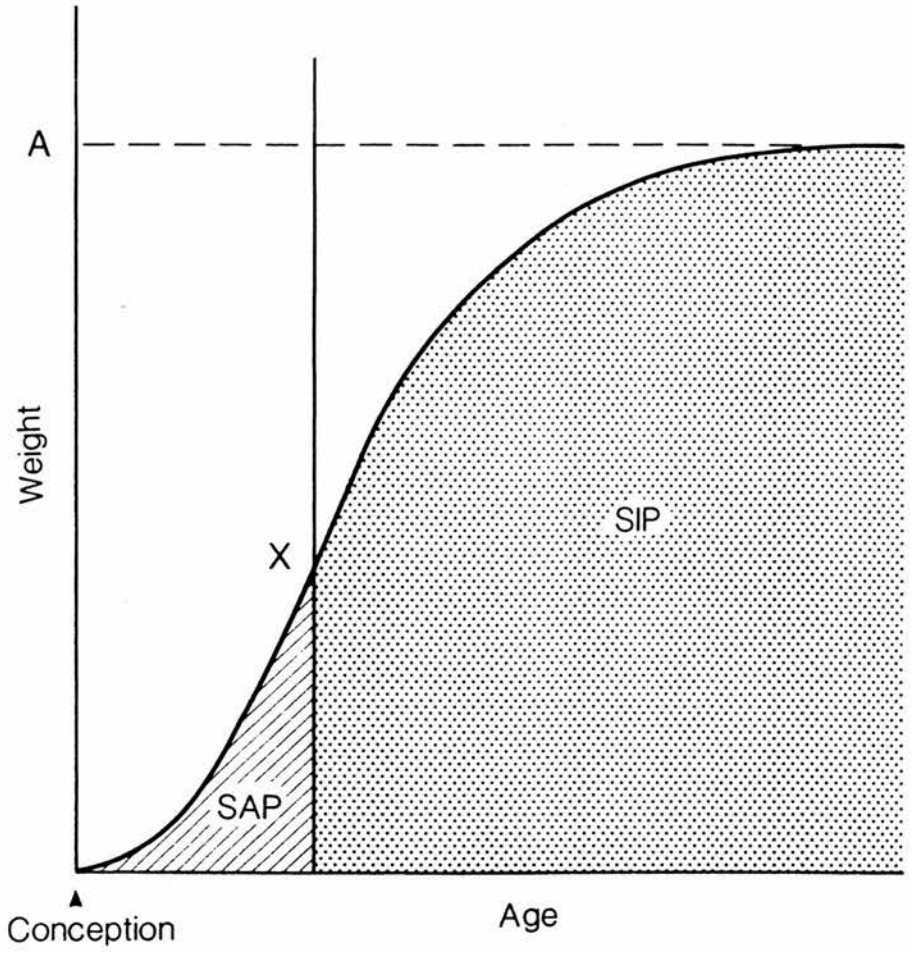


Figure 1.1.2.2.1 Idealized growth curve. (A = mature weight, X = inflection point, SAP = self accelerating phase, SIP = self inhibiting phase)

McCarthy and Bakker, 1979). Therefore, the benefit of larger animal would be lost due to a lower relative rate of growth. Alternatively, animals with a higher relative growth rate but the same mature weight could be selected. In this case the mature weight is achieved faster which improves the intrinsic efficiency of the animal. The second tactic means changing the shape of the growth curve by exploiting any flexibility in the genetic relationship between mature size and the rate of maturing. Altering the shape of the growth curve can also be advantageous for other reasons. For instance, dystocia problems could be alleviated by decreasing birth weight relative to dam size and also the genetic antagonism between producing rapid and efficient early growth in the young for slaughter from dams which are small and have low maintenance costs could be relieved (Fitzhugh, 1976).

Most evidence from traditional, single trait selection experiments indicates that breeding for increased body size does not alter the basic shape of the growth curve but merely leads to animals with a larger mature weight or a greater absolute growth rate over the entire growth curve (Roberts, 1961; Eisen et al, 1969; Timon and Eisen, 1969). This is illustrated by Figure 1.1.2.2.2. Roberts (1961) noticed that his two largest strains of mouse both selected for high 6 week weight, RCL and NF strains, reached the same mature weight but at vastly different times: RCL at approximately six months age and NF at one year. Thus, the basic correlation between mature weight and relative growth rate is not fixed.

Since single trait selection was usually unsuccessful at changing the relative growth rate, several workers have examined the efficacy

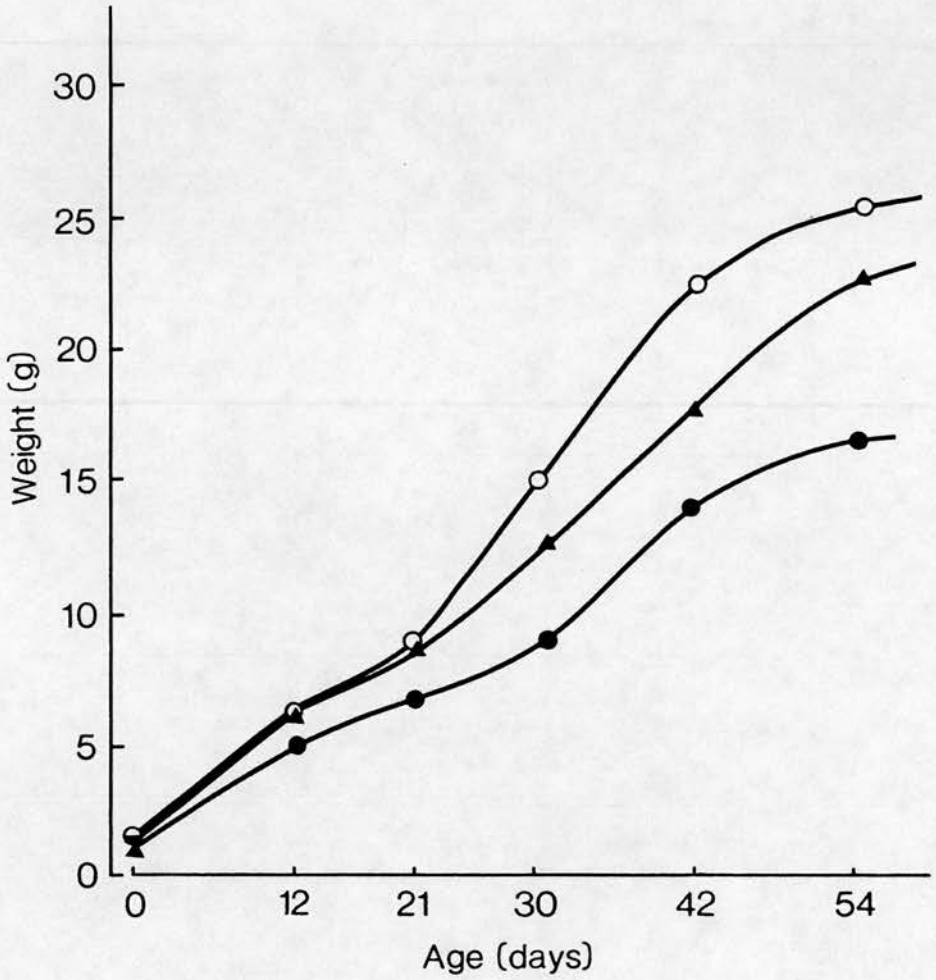


Figure 1.1.2.2.2 Growth curves of mice selected for high (○) and low (●) 6 week body weight and of the unselected control (▲).

(from Lang and Legates, 1969)

of a multi-trait approach. McCarthy (1971) selected mice for a combination of 5 and 10 week weights. The line bred for high 5-low 10 week body weight (H5,L10) was approximately 10% heavier at 5 weeks but 7% lighter at 10 weeks than the line selected for low 5-high 10 week body weight (L5,H10). Thus, the shape of the growth curve was significantly altered. Similarly, Wilson (1973) obtained an alteration in the shape of the growth curve by attempting to increase the ratio of early postweaning gain to total postweaning gain in mice (21-42 d gain / 21-63 d gain). The change was due to a decrease in 63 d weight with a constant 42 d weight rather than an increase in early postweaning gain together with unchanged 63 d weight. McCarthy and Bakker (1979) examined the relationship between maturing rate and mature size by three types of selection. First, they bred mice for high or low body weight at one age in a traditional type of single trait experiment. Predictably, large variations in mature size were evident but the rate of maturing was stable which left the shape of the growth curve unchanged. Secondly, selection for both 5 and 10 week weights in opposite directions (repeating McCarthy's 1971 trial), resulted in a minimal difference in mature weight but a large difference in the rate of maturing and therefore in the form of the growth curve. Finally, growth curves were compared between mice selected either for the same 5 week weight but diverging 10 week weights or for the same 10 week weight but diverging 5 week weights. This combined index produced alterations in both the rate of maturing and in mature weight. Using a multi-trait selection index has not been universally effective in altering the form of the growth curve. Four lines of mice bred for all high low combinations of 12-21 d gain and 51 d body weight responded in the desired direction for 51

d weight but 12-21 d gain was opposite to the direction of selection in the low high and high low lines (Harvey, 1972). Thus, two trait selection indexes do not always affect the form of the growth curve. However, overall this method of selection was moderately successful although only small rates of change were achieved.

Analysis of growth curves is invaluable for information on whole body growth characteristics but they do not reflect accretion of individual components of the body which is of relevance when extrapolating to selection in domestic species. Therefore, examination of body composition is crucial in assessing the efficacy of selection criteria.

1.1.3 THE EFFECTS OF GENOTYPE UPON BODY COMPOSITION

1.1.3.1 Changes in Fat Proportion

Selection for high body weight or rapid postweaning gain leads to increased weight of body components (Biondini, Sutherland and Haverland, 1968) but they often alter disproportionately. A number of studies showed an increase in the percentage of fat in mice selected for rapid postweaning gain (Biondini et al, 1968; Robinson and Bradford, 1969 and Timon, Eisen and Leatherwood, 1970) or for high body weight (Fowler, 1958; Romsos and Leveille, 1974; Sutherland, Biondini and Ward, 1974 and Baker, Carter and Cox, 1979). However, other studies showed no change in the proportion of body fat (Lang and Legates, 1969; Stanier and Mount, 1972; Romsos and Leveille, 1974). Timon and Eisen (1970) concluded that a major consequence of selection for postweaning gain was increased food

consumption. In a later study with the same lines of mice, body composition between lines was compared on ad libitum and restricted feeding (Timon et al, 1970). When differences in appetite were excluded the difference in the proportion of fat disappeared. Therefore, it was suggested that an increase in fat content is a consequence of a raised capacity for food consumption.

It is interesting to contrast the proportions of fat in the body between mice selected for high body weight and mice possessing the major gene, hg/hg, for rapid postweaning gain. Mice with the double recessive hg/hg genotype gain 30 to 50% more weight from 3-6 weeks age and have a greater mature weight than Hg/- contemporaries (Bradford and Famula, 1984). These differences are comparable with those mice divergently selected for postweaning weight gain or body weight. However, mice with the double recessive genotype increase their body weight by proportional growth (Calvert, Famula and Bradford, 1984; Calvert, Famula, Bernier and Bradford, 1985) and not by an exaggerated response in lipid accretion. Conversely, obese Zucker rats which consume much more food and are heavier than their normal counterparts deposit a much greater percentage of fat than mice bred for high body weight (Radcliffe and Webster, 1976). In absolute terms however, Zucker "fatties" deposit exactly the same amount of protein as lean Zucker rats (Radcliffe and Webster, 1976). When food intake was restricted to the level of their lean counterparts, obese Zucker rats still had a greater proportion of fat but less protein whereas restricted feeding in mice selected for high postweaning gain caused the differences in fat percentage to disappear and the proportion of other body components was the same (Timon et al, 1970). Therefore, genetic variation in the percentage

of body fat in laboratory animals is very wide. The two extremes are ob/ob Zucker rats which have a very high proportion of body lipid and hg/hg mice that have a normal proportion of body fat but are extremely large.

1.1.3.2 Changes in the Proportion of Protein

Genetic selection does not appear to change the proportion of protein as much as it changes fat. Timon et al (1970) found no difference in percentage protein between mice selected for high 3-6 week weight gain and control animals. Most studies in which selection for high postweaning gain or high body weight (section 1.1.3.1) lead to an increase in the proportion of body fat reciprocally reduced the proportion of body protein (Palmer, Kennedy, Calverley, Lohn and Westwig, 1946), However, percentage protein as a proportion of fat-free body weight was not altered. These data led to the conclusion that it may be very difficult to change the composition of the fat-free carcass (Timon et al, 1970). These findings were reinforced when direct selection for increased protein weight at 70 days age was unsuccessful (More O'Ferrall and Timon, 1970). On the other hand, McLellan and Frahm (1973) set up a two-way selection study for weight of the hindleg muscle system in mice and the proportion of muscle was diminished in the low line although the percentage of muscle was not altered between high and control lines. Therefore, if hindleg muscle is taken as representative of whole body protein it is perhaps easier to reduce protein proportions than increase them. In another mouse study an attempt was made to change the lean mass but not body weight (Sharp et al, 1984). Fat-free mass was estimated using the index [10 week

body weight minus (8 X gonadal fat pad weight)]. Despite the total protein weight diverging by 22%, between the high and low lines, this change was due to alterations in total body weight. Therefore, the primary aim of a higher proportion of lean mass was not achieved. However, the rate of lean gain was altered and larger mice with proportional growth of all body components were obtained. This is an improvement upon conventional experiments for body weight selection where increased body weight is associated with an increased proportion of fat. Interestingly, this pattern of growth was very similar to that of mice with the double recessive hg/hg genotype for rapid postweaning gain where increased body weight is also due to proportional growth (Calvert et al, 1984; Calvert et al, 1985). In conclusion, it appears to be more difficult to alter the proportion of protein than fat by genetic means.

1.1.3.3 Alterations in Fat and Protein Distribution

Few studies have examined the effect of genetics on fat and protein distribution. Mice selected for high body weight have an increased percentage of fat (Allen and McCarthy, 1980). Kidney and gonadal fat depots were shown to increase disproportionately in these mice (Allen and McCarthy, 1980) but there is little other evidence on fat distribution in laboratory animals. There is ample evidence that fat distribution differs between breeds of sheep (Seebeck, 1968; Donald, Read and Russell, 1970; Kempster and Cuthbertson, 1977) and cattle (Pomeroy and Williams, 1974; Kempster, Cuthbertson and Harrington, 1976) but not in pigs (Richmond and Berg, 1971; Martin, Freeden, Weiss and Carson, 1972).

There have been no studies on protein distribution in mice divergently selected for body weight and postweaning gain but in obese Zucker rats a greater proportion of the protein deposited was as skin protein and less as muscle protein (Radcliffe and Webster, 1976). Direct selection for altering the hindleg muscle system weight in mice was not very successful and the proportion of muscle was not increased above control levels in the high line although it was reduced in the low line (McLellan and Frahm, 1973).

1.1.4 AGE OF SELECTION AND ITS EFFECT ON BODY COMPOSITION

It has been noted above that selection for body weight has a profound effect upon body composition. The following section is also concerned with body composition but discusses how the age at which an animal is selected can have a large effect upon body composition.

A concensus emerging from a number of studies is that mice selected at an earlier age will become fatter than those selected later, nearer to puberty (Fowler, 1958; Hull, 1960; Hayes and McCarthy, 1976; Baker, Carter and Cox, 1979; Allen and McCarthy, 1980). Growth in young animals is predominantly of non-fatty tissue. Therefore, it was originally thought that selection at younger ages would largely increase non-lipid tissue. Conversely, it was thought that selection at older ages would result in greater amounts of fat accretion (Hull, 1960). However, the opposite results were found; animals selected younger became fatter. Hayes and McCarthy (1976) proposed a model suggesting that selection of young animals chooses those that have bigger appetites. This will

lead to fatness nearer to maturity. On the other hand, fat deposition will have begun when animals are selected at an older age. The energy cost of producing and maintaining a similar weight of lean tissue is much less than fat (Webster, 1980; section 1.2.3). Therefore, selection at a later age will tend to pick out those animals accreting more lean than fat. Hence, the leaner animals will also be larger. This model is supported by a study where animals were selected for six week body weight on either ad libitum or restricted food consumption. If the model is correct then, mice on restricted feeding should be selected due to differences in body weight associated with alterations in efficiency and not connected with changes in food intake. After establishment of high body weight lines on both feeding regimes those selected on restricted feeding were tested under ad libitum conditions: as well as growing as fast as ad libitum counterparts they were also slightly leaner (Falconer and Latyszewski, 1952). Thus, age of selection is important since it will determine whether differences in body weight are caused by variation in food intake or by differences in efficiency with subsequent varying effects upon body composition.

1.1.5 GENETICS, FOOD INTAKE AND FOOD CONVERSION EFFICIENCY

Having established that body growth and body composition are altered by selection it is also important to understand how these changes occur. This section is concerned with the relationship between these criteria and the amount of food ingested and its fate.

It is well established that selection for high body weight or postweaning gain in mice will increase food consumption above control levels (Fowler, 1962; Rahnefield, Comstock, Boylan and Singh, 1965; Lang and Legates, 1969; Timon and Eisen, 1970; Sutherland, Biondini, Haverland, Pettus and Owen, 1970; Roberts, 1974; Hayes and McCarthy, 1976; Eisen and Bandy, 1977; Kownacki and Jezierski, 1980; Roberts, 1981; Graham and McCarthy, 1982). Conversely, food intake is lowered in mice selected for low body weight or postweaning gain. In addition, selection for high food intake increased growth rate (Sutherland et al, 1970). Thus, a high genetic correlation between food intake and body weight seems to exist. It was suggested that the same genes affected both food intake and body weight (Sutherland et al, 1974) and that consequently, changes in body weight were mostly a function of food consumption. However, a large body of evidence opposes this conjecture. Falconer and Latyszewski (1952) selected mice for high 6 week weight on either ad libitum or restricted feeding (75% of ad libitum level). Once the lines were established each line was tested on the alternative feeding regime. Whilst the ad libitum selected line showed no improvement in growth over controls when placed on restricted feeding, the body weight of the line selected on restricted feeding when fed ad libitum increased to almost the same levels as the ad libitum selected lines. Therefore, differences in growth rate established when variation in food consumption was damped also lead to alterations in food intake when appetite was allowed to be expressed whereas differences in growth established when appetite could be expressed were abolished when food intake was restricted. This implies that the effect of selecting for body weight is primarily upon genes affecting body

weight with secondary changes in food intake (McCarthy, 1979). There are other similar studies which support this (Falconer, 1960; Dalton, 1967; Stanier and Mount, 1972; Meyer and Bradford, 1974; Petersen and McCarthy, 1981; McPhee and Trappett, 1987).

Many of these experiments support the hypothesis developed in pigs that selection of animals for body weight on a set level of feeding would favour those animals that directed more metabolizable energy to protein and less to fat accretion and hence, were more efficient (Kielanowski, 1968; Fowler, Bichard and Pease, 1976). For instance, McPhee and Trappett (1987) selected mice under restricted feeding conditions and then fed the animals ad libitum. Energy was partitioned in favour of more protein deposition in comparison with animals selected under ad libitum conditions.

Once food has been ingested there are many ways in which it can be partitioned and so the efficiency with which body weight is gained can be drastically altered. Food conversion efficiency (FCE) is the term used to describe how well food is laid down as body weight and is taken as weight gain/food intake. FCE is made up of many components.

Firstly it depends upon food digestibility. Of the few genetic studies that have examined digestibility there is little evidence of any alteration in this component. Stanier and Mount (1972) found no differences in food digestibility between their mice divergently selected for mature body weight. Even though Fowler (1962) showed her fast-growing strain absorbed more protein and fat, less carbohydrate was taken up from the gut with the net result that

energy digestibility was the same for both strains. In addition, food digestibility between lean and obese Zucker rats was not found to differ (Deb, Martin and Herschberger, 1976). In fact, Blaxter (1968) stated that there was very little difference even between species in food uptake from the gut. Nevertheless, an experiment in which rats were selected for rate of gain on a lysine-deficient diet showed that more efficient food utilization by the rapidly growing strain was at least partly due to an increased protein digestibility. However, this study was more concerned with nutrient deficiency than the genetics of growth. It is probable that the genetic differences in efficiency of growth rate are rarely due to alterations in feed digestion or absorption.

Secondly, FCE is affected by the division of energy into maintenance and growth. Many studies have shown that the gross efficiency of food utilization was greater in mice bred for increased body weight or postweaning gains and vice versa for low lines (Fowler, 1962; Lang and Legates, 1969; Timon and Eisen, 1970; Sutherland et al, 1970; Meyer and Bradford, 1974). The gross efficiency is calculated from tissue weight gain and intake directed towards the tissue's requirements for maintenance as well as for growth whereas net efficiency only takes account of intake for tissue growth and excludes intake for tissue maintenance. However, it was suggested that there were no improvements in net efficiency (Timon et al, 1970) but that gross efficiency was improved simply because high selected animals consumed more per unit time than unselected animals. This would give high lines more energy above maintenance requirements and thus a greater availability of energy for growth (Sutherland et al, 1974).

Therefore, it was necessary to distinguish between the division of metabolic energy into maintenance and growth.

The conclusion from the majority of studies where maintenance energy has been examined was that both basal metabolic rate (BMR) and maintenance energy costs were slightly decreased in high selected lines (Kownacki, Keller and Gebler, 1975; Kownacki and Keller, 1978; McCarthy, Petersen, Graham and Willeke, 1978-79; Kownacki and Jezierski, 1980; McPhee, Trappett, Neill and Duncalfe, 1980). This implies that lower maintenance costs were one reason for increased efficiency. For instance, Kownacki and Keller (1978) found the basal metabolic rate was 8.5% lower in mice bred for high postweaning gain ($p<0.01$). Mice with the major gene, hg/hg, for rapid postweaning gain also had slightly decreased maintenance requirements (Bernier, Calvert, Famula and Baldwin, 1986). In mice selected for high postweaning gain (Canolty and Koong, 1976) and in lean and obese Zucker rats (Deb et al, 1976) no differences were found in maintenance energy needs between lines or types. Conversely, other groups concluded that mice and rats bred for high postweaning gain had higher maintenance requirements than their low strain counterparts (Brown and Frahm, 1975; Rios, Nielsen, Dickerson and DeShazer (1986). Therefore, lower basal metabolic rate/maintenance energy costs may permit more efficient utilization of food nutrients in mice bred for high body weight. However, individual cases differ and there are several exceptions that do not fit this pattern.

The remaining energy that can be directed towards growth is divided between the various body components. The most relevant

partition in this situation is between protein and fat. In mice selected for postweaning gain, high and control animals showed no change in the lean energy deposition coefficient but the fat energy deposition coefficient was greater in the high line (Canolty and Koong, 1976). The energy deposition coefficient is the product of the proportion of metabolizable energy available for gain and efficiency of gain. This suggests that the high selected mice either used a higher proportion of metabolizable energy available for gain for fat deposition or the energy directed to fat deposition was utilized more efficiently.

In conclusion, improvements in feed conversion efficiency do not seem to be caused by alterations in digestability of nutrients but appear to be a function of increased food consumption, altered maintenance requirements and growth energy partition. The efficiency of individual tissue deposition may also contribute towards growth differences.

1.1.6 GENETIC DIFFERENCES IN BIOCHEMICAL AND METABOLIC TRAITS

Given that selection for growth affects energy partition and maintenance requirements it is likely that metabolic processes such as protein turnover will also be altered. The involvement of hormones in controlling these processes may also change with selection and some hormones have therefore been reviewed with respect to genotype. It is particularly useful to examine changes in hormone concentrations because if a sufficient difference was evident in a certain hormone between selected animals then this would provide an ideal biochemical marker that was easy and quick to

measure but not expensive.

1.1.6.1 Hormonal Differences

Knowledge concerning the interaction of hormones, body size and growth due to genotype is sparse and so information on only the two most studied hormones will be presented here.

a) Growth Hormone

A significant positive correlation was found between growth hormone (GH) levels and relative growth rate in broiler chickens selected for high growth rate (Burke and Marks, 1982). Alternatively, in three broiler strains selected for fast growth rate, the strain which grew the fastest had the lowest GH concentrations (Stewart and Washburn, 1983). The fastest growing strain was also the fattest. Goddard, Wilkie and Dunn (1988) found no correlation between plasma GH concentrations and the rate of growth in chickens. Therefore, GH may or may not be involved in regulating rate of weight gain in chickens. Obviously these studies were not very comprehensive and factors other than absolute growth rate may matter, for example body composition.

The situation in pigs is slightly clearer but again information is sparse. Larger amounts of GH were secreted into the blood of swine selected for rapid rate of gain compared with slow growing pigs (Baird, Nalbandov and Norton, 1952). Additionally, growth hormone levels were lower in Ossabaw pigs compared with larger Yorkshire pigs (Kasser, Martin, Gahagan and Wangsness, 1981).

Ossabaw pigs are a feral breed that have a much slower growth rate than the domestic Yorkshire breed. Newborn Ossabaws also had higher carcass lipid and dry matter concentrations than newborn Yorkshire piglets. Thus, it is indicated that GH levels may be positively correlated with growth rate in swine.

This trend also holds true in laboratory animals. Comparison of six different strains of rat showed that those with the highest body weight also had the highest circulating GH concentrations (Esber, Menninger and Bogden, 1974). In addition, Snell dwarf mice which only grow to approximately one third the size of normal littermates, lack GH (Bates and Holder, 1986). Upon injection of GH their growth rates increased considerably (Holder and Wallis, 1977). Further evidence implicating GH involvement in regulation of body size through genotype comes from a study with mice selected for large and small size (Pidduck and Falconer, 1978). High, control and low line mice selected for body weight were genetically hypophysectomized by crossing with Snell dwarf mice (dw/dw). In the absence of GH the different strains still grew at different rates. This showed that GH status was not the only factor contributing towards growth differences. However, the reduction in growth rate was more pronounced in the high than the control group and was less evident in the low line than the control. In addition, the small strain of hypophysectomized mice was less sensitive to a set level of GH administration than the large line. This implies that the growth differences were at least partly due to GH. Although the relationship is not a simple one, it appears that GH is at least partly responsible for the genetic differences in growth rates in a number of species. The

anabolic actions of GH are at least partially mediated via insulin-like growth factors (IGFs) (Isaksson, Eden and Jansson, 1985). However, examination of IGF-I concentrations upon GH administration to sheep showed elevated IGF-I levels without increased growth rates (Pell, Blake, Buttle, Johnsson and Simmonds, 1987). Therefore, the endocrine role of IGFs appears to be equivocal. Unfortunately, IGF-1 concentrations have not yet been examined in animals of different genetic types.

b) Thyroid Hormones

There is no evidence that genetic differences in growth rate in swine are caused by differences in thyroid hormones. One study could not distinguish between Pietrain and Large White pigs on the basis of thyroid weight, total tri-iodothyronine (tT_3), total thyroxine (tT_4), free tri-iodothyronine (fT_3), free thyroxine (fT_4) or reverse tri-iodothyronine (rT_3) (Bocklen, Flad, Muller and von Fabler, 1986). However, both Pietrain and Large White pigs grow comparatively quickly and so, if thyroid hormone parameters affected growth rate, any differences would not be expected to show up well between these two breeds. In addition, no change was found in thyroid gland weight between pigs bred for rapid and slow growth (Baird et al, 1952). On the other hand a single study in chickens indicated that of three broiler strains selected for rapid growth rate, the most rapidly growing strain had the highest T_3 concentrations (Stewart and Washburn, 1983). There is conflicting evidence in laboratory mammals as to the involvement of thyroid hormones in the genetic regulation of growth rate. Esber et al (1974) could find no connection between

body weight and T_4 levels in six different rat strains chosen for their differing body weights. Alternatively, Snell dwarf mice which lack GH are also deficient of T_4 (Bates and Holder, 1986). Upon T_4 treatment the growth rate of Snell dwarf mice was increased with a concomitant increase in serum somatomedin level (Holder and Wallis, 1976). Therefore, thyroid hormones are connected with growth in mice. In rats there were significantly lower T_3 and T_4 levels in the faster-growing Sprague Dawley strain with higher FCE compared with the Wistar strain (Kuhn, Bellon, Huybrechts and Heyns, 1983). As mentioned in section 1.3.3.1 thyroid hormones may be connected with food intake which was, however, equal in Sprague Dawley and Wistar rats. Therefore, despite thyroid hormones undoubtedly affecting body weight as demonstrated by Snell dwarf mice, their influence upon genetic aspects of growth is not clear.

1.1.6.2 Protein Metabolism

Few experiments have examined alterations in protein turnover and metabolism in relation to genetically induced differences in growth and from them no single conclusion has been reached. One of the first studies discovered a positive correlation in mice between body weight, carcass weight and carcass nitrogen with protein metabolic activity at four weeks but a negative correlation at six weeks of age (Gall, Kyle, Rogler and Anderson, 1967). Protein metabolic activity was equated to the distribution of α-amino isobutyric acid (a non-metabolizable amino acid) between tissue and serum to give a measure of the capacity of a tissue to incorporate amino acids into cells. Gall et al (1967) concluded that a genetic relationship

existed between protein metabolic activity and weight gain at a particular time. They argued that this relationship was a better measure of growth processes than the relationship between gain and body weight. However, the validity of the distribution of α-amino isobutyric acid as a measure of protein metabolic activity is questionable.

Using better methods, growth rates were compared with skeletal muscle fractional breakdown rates in a fast growing albino strain (CFY) (Bates and Millward, 1978) and a slow growing hooded strain of rat (Millward, Garlick, Stewart, Nnanyelugo and Waterlow, 1975). The faster growing strain had a much lower protein turnover rate than the slow growing hooded strain. This was especially noticeable between the youngest animals compared (25 d old) where turnover rates in the CFY strain were only half those in the slow growing strain (Millward, 1978). Thus, between strains it appears that animals with higher growth rates have lower protein turnover rates. It should be noted that the studies on the two strains were performed at different times which may have affected the results.

When growth and protein turnover rates were compared within strains the relationship differed and the rate of skeletal muscle protein degradation was proportional to growth rate (Bates and Millward, 1978). Similarly, both skeletal muscle and liver protein synthesis rates were reduced in slow growing Snell dwarf mice compared with their normal littermates (Bates and Holder, 1986). Therefore, growth rate differences between and within strains seem to be affected by alteration of metabolic processes in different ways.

The rate of protein synthesis was consistently greater in liver and kidney of low than high line mice (Priestley and Robertson, 1973). In addition, a similar pattern emerged for whole body protein synthesis and degradation rates in laying and broiler chickens. The slower growing layers had higher protein turnover rates than larger, faster growing broilers (Saunderson and Bryan, 1985; Muramatsu, Aoyagi, Okumura and Tasaki, 1987). Across different species ranging in size from a 20g mouse to over a 600kg cow it has been noted that whole body protein turnover decreases with increasing body mass (Garlick, 1980b). Thus, the genetics of growth do appear to affect protein metabolism but not necessarily in the direction that would seem most logical at first glance. However, from a different viewpoint it is rational that faster growing strains should have lower protein turnover rates because it would necessarily follow that protein accretion would be greater from the same amount of energy intake in these lines. Hence, a lower rate of protein metabolism may be one of the methods by which genetic increases in body weight and growth arise.

1.1.7 CHANGES IN CELL STRUCTURE AND NUMBER WITH GENOTYPE

Protein metabolism is markedly affected by genotype and the total amount of protein can be considerably increased by selection for large body size. Therefore, it is reasonable to expect structural differences in the largest protein-containing organ: skeletal muscle. Muscle size can be altered by changing the number of fibres and/or the size of each fibre. Normally, prenatal growth is characterized by hyperplasia and postnatal growth by hypertrophy (Goldspink and Kelly, 1984). Bowman and McLaren (1970) counted

total number of cells of three and a half day post coitum mouse embryos from high and low body weight selected lines. No difference was found in the number of cells per embryo. This disagrees with the hypothesis that divergence in adult body weight is caused by a steady difference in the rate of cell division throughout development (Bowman and McLaren, 1970). Hence, non-uniform rates of cell division occur sometime after the early developmental stage to give rise to superior body sizes. The first examination of muscle structure between unselected control mice and mice selected for rapid postweaning gain between 3 and 6 weeks of age concluded that the 30% difference in body protein was caused by a difference in cell number rather than cell size (Robinson and Bradford, 1969). Similarly, in mice selected for high or low 5 or 10 week body weight, fibre number was correlated with muscle size in seven different muscles (Byrne, Hooper and McCarthy, 1973; Hanrahan, Hooper and McCarthy, 1973b). However, the fibre diameter was also altered and compared to controls was greater in the high lines but smaller in low lines. Interestingly, when the muscle structure of the same lines was compared at a standard weight rather than age, there were more but smaller fibres present in the high line muscles (Hooper and McCarthy, 1976). Thus, selection for high body weight or rapid postweaning gain tends to result in muscles with a greater number of larger fibres. Differences in the rate of cell division are the primary change followed by marked hypertrophy. Contrary to these findings, Luff and Goldspink (1970) discovered no relationship between fibre number and muscle size when they compared four unrelated mouse strains. It is worth noting that direct selection for biceps fibre number in mice had produced no effect after three generations in either body weight, biceps weight or cell mass as

well as fibre number (Graham and Hooper, 1982). If anything, fibre number was slightly greater in mice selected for low fibre number. However, three generations is a short time interval in which to expect genetic differences to be manifest.

Muscle ultrastructure has also been examined. Hooper (1976) observed that high line mice had larger muscle fibres due to an increase in the number of sarcomeres per fibre. However, there was no alteration in the size of the sarcomeres. From this he suggested that longitudinal muscle fibre growth is affected by genotype in two ways: between different species myofilament length is changed and within species fibre growth differs due to the formation of additional sarcomeres. Hooper and Hurley (1983) confirmed this when they found differences in the number of constituent sarcomeres and myofibrils between strains which caused differences in muscle fibre dimensions.

It emerges from this review of conventional selection procedures that in addition to changes occurring for example in body weight, composition and growth rate, biochemical and endocrinological differences are evident. In the following section factors influencing protein metabolism are examined with a view to relating differences in genotype to differences in protein turnover.

1.2 PROTEIN METABOLISM AND TURNOVER

Protein accretion in an animal inevitably reflects the rates of protein synthesis and degradation. Selection may alter either process to bring about different growth rates. Both processes are reviewed and discussed in this section bearing in mind points at which regulation may occur.

1.2.1 REGULATION OF PROTEIN SYNTHESIS

Since protein synthesis involves so many different steps and factors there are many stages at which it can be regulated. Control at both transcription and translation occurs.

Regulation of transcription tends to influence the synthesis of very specific proteins rather than all proteins. For example, thyroid hormones have been shown to affect the expression of the GH gene in rats. Hypothyroidism was associated with a fall in pituitary GH mRNA levels and was accompanied by a decrease in serum GH and pituitary GH content. Upon treatment of hypothyroid animals with T_3, GH mRNA levels were stimulated to values above those seen in euthyroid controls (Wood, Franklyn, Docherty, Ramsden and Sheppard, (1987).

As well as transcriptional control, the rate and efficiency of translation can differ. There are three main stages in translation: initiation when the ribosome and starting amino acyl-tRNA are attached onto mRNA, peptide chain elongation when amino acids are

attached as ribosomes traverse mRNA and finally termination involving release of the new polypeptide chain and tRNA from mRNA.

An overall alteration in the rate of protein synthesis is normally due to a change in ribosomal number and activity. A change in ribosomal activity usually indicates a change in the rate of initiation. For instance, iron deficiency in reticulocytes results in a rapid fall in protein synthesis due to disaggregation of polysomes (London, Clemens, Ramu, Levin, Cherbas and Ernst, 1976). Similarly when one or more essential amino acids is omitted from the reticulocyte culture medium, complete polysome disaggregation occurs in less than one hour (Pain and Clemens, 1980).

Alterations in the nutritional status often affect RNA parameters with accompanying changes in protein synthesis (eg Millward, Nnanyelugo and Garlick, 1974a,b; Li and Goldberg, 1976; McNurlan, Pain and Garlick, 1980). Hormonal changes elicited by nutritional deficiencies or excesses may bring about these alterations in synthesis. For instance, hydrocortisone has been shown to bind to some tRNA species (eg phenylalanyl-tRNA) with greater affinity than others. This was shown to hinder amino acid incorporation into the peptide (Chin and Kidson, 1974). Additionally, it has been suggested that steroids act at a site on the endoplasmic reticulum and affect the proportion of bound to unbound ribosomes which in turn may influence cellular protein production (Thompson and Lippman, 1974).

Thus, both transcriptional and translational regulation appears to allow both the rate of synthesis of all proteins and of individual

proteins to be altered in response to nutritional and hormonal stimuli.

1.2.2 PROTEIN DEGRADATION

The mechanism and control of protein degradation is not well understood even though the different conditions which initiate alterations in proteolysis are well documented. Protein breakdown occurs continuously which may seem energetically uneconomic. However, sustained protein degradation serves several necessary functions within the body. Firstly, abnormal proteins produced from mutations, errors in gene expression and denaturation can be degraded and so prevent deleterious effects in the cell. It has been pointed out that this type of protein degradation would be especially important in slow-growing tissues (ie most mammalian tissues) since unlike fast-growing tissues, deleterious proteins cannot be quickly diluted out by rapid growth (Goldberg and Dice, 1974). Secondly, constant underlying proteolysis and protein turnover allows for ready adaptability to the environment. This is particularly important for regulatory enzymes where a rapid rate of breakdown will allow enzyme levels to change quickly in response to hormonal and nutritional alterations. It has been noted that the rate of degradation correlates well with the regulatory role of a particular enzyme. In rat liver, those enzymes catalyzing rate-limiting or the first steps in a metabolic pathway have the shortest half-lives in contrast to enzymes in less crucial positions that have the slowest breakdown rates (Goldberg and Dice, 1974; Mayer, 1978). These enzymes are often large and several years ago it was believed that structural properties such as size and acidity

were of primary importance in determining the rate of degradation of a protein. However, this has since been contradicted and structure and conformation are no longer thought to influence protein breakdown despite the strong correlation between these characters (Mayer and Doherty, 1986). This regulatory proteolysis is selective and requires a mechanism for identifying defective proteins or individual enzyme proteins for accelerated degradation. Another example of specific proteolysis, although quantitatively much less important, is post-translational modification of proteins eg proinsulin → insulin is a very specific type of peptide bond cleavage.

The cell also has pathways for gross, non-selective protein degradation which can liberate amino acids for energy metabolism in times of nutrient deprivation.

Several mechanisms for protein degradation have been suggested all of which hypothesize a number of degradative pathways to explain the existence of selective and non-selective protein degradation. The lysosomal system has a major role in protein catabolism and there is evidence that almost all enhanced proteolysis due to nutrient deprivation is via lysosomal autophagy (Mortimore, Neely, Cox and Guinivan, 1973; Neely, Nelson and Mortimore, 1974). Lysosomal autophagy has also been implicated in basal protein degradation (Mortimore and Ward, 1981). Thus, it appears that general proteolysis of all protein types occurs via lysosomal degradation. Much evidence for non-lysosomal protein breakdown is also available (McElligott and Dice, 1983; Mayer and Doherty, 1986) with indications of the existence of at least one cytosolic degradation

system. One of the most studied non-lysosomal proteolytic mechanisms is an ATP-dependent system in the reticulocyte, first observed by Schweiger, Rapoport and Scholzel (1956). This system was noticed to be very active in degrading abnormal proteins (Rabinovitz and Fisher, 1962; 1964). Separation and examination of the reticulocyte lysate revealed two fractions (ATP-dependent Proteolysis Factors I and II, APF I and II) both necessary for protein breakdown. Subsequently, APF I was identified as being ubiquitin (Wilkinson, Urban and Haas, 1980) and it was assumed that conjugation of ubiquitin to proteins served as a recognition signal for the selectivity of degradation of intracellular proteins (Hershko, Ciechanover, Heller, Haas and Rose, 1980). A cyclical process involving conjugation of ubiquitin to breakdown proteins and then endo-proteolytic attack to produce short peptides with release of ubiquitin for reuse as a final stage has been proposed (Hershko and Ciechanover, 1982) (Figure 1.2.2.1).

Recently it has been reported that the amino acid at the N-terminal of a protein chain helps determine the half-life of the protein (Bachmair, Finley and Varshavsky, 1986). The different amino acids give half-lives ranging from more than 20 hours to about 2 minutes in a yeast species which covers the whole range of known protein half-lives in these cells. Thus, it seems that ubiquitin may not act as the primary or only recognition signal for degradation although after identification of the N-terminal, attachment of ubiquitin molecules may be the next step.

ATP-dependent proteolytic systems other than the ATP-ubiquitin process have been described, mainly in E. coli. In addition,

Figure 1.2.2.1

1. Activation of the carboxyl terminal glycine of ubiquitin and its intermediary binding to the activating enzyme (E_1).

2. Conjugation of several molecules of activated ubiquitin to ε-NH_2 lysine groups of the substrate proteins.

3. Breakdown of conjugates by an endoprotease acting on the protein substrate moiety.

4. Release of short peptides and free ubiquitin by a specific amidase that cleaves the isopeptide linkage.

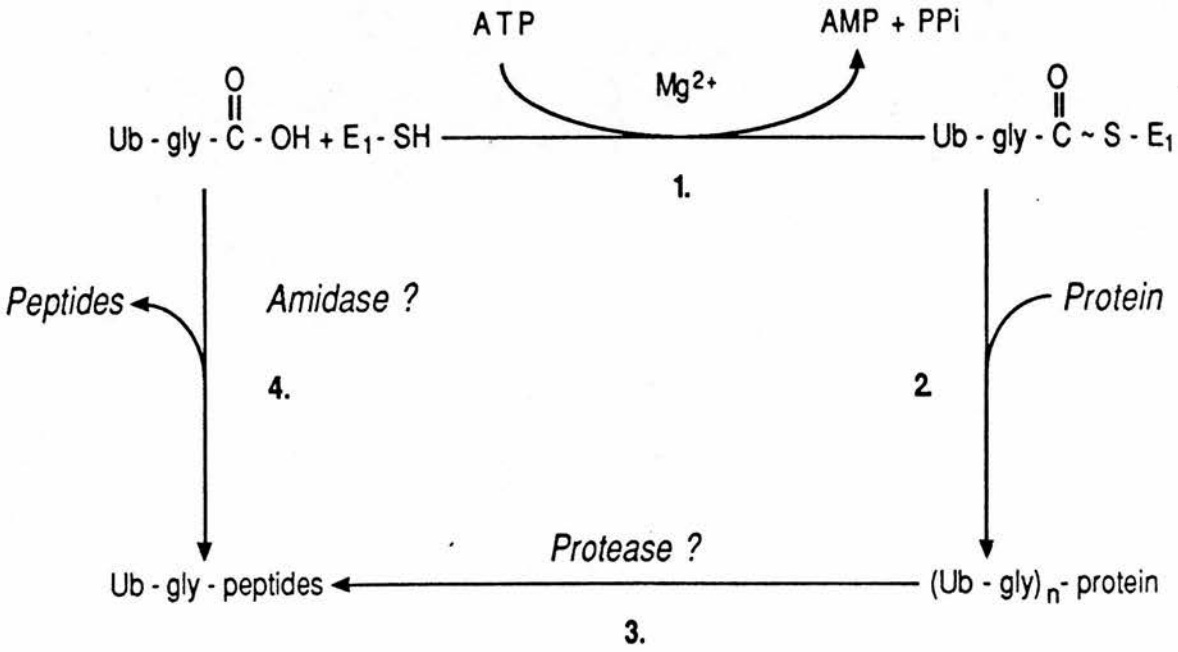


Figure 1.2.2.1 Proposed pathway of ubiquitin-mediated protein degradation.

(Hershko and Ciechanover, 1982)

calcium-dependent proteolytic systems have been discovered and it has been proposed that they relate to the cytoskeleton (Murakami, Hatanaka and Murachi, 1981; Croall and De Martino, 1983).

All the degradation systems mentioned so far are for intracellular breakdown of proteins. Extracellular degradation also accounts for a considerable part of whole body breakdown in processes such as loss of intestinal cells and digestive enzymes, red cell turnover, skin and hair loss . This is not degradation in the sense of splitting proteins into amino acids but parts or whole proteins are effectively "lost" from the body and so should be regarded as degradation. This type of protein "degradation" can account for a considerable proportion of total protein degradation.

Since there are many mechanisms of protein degradation then there are also many opportunities for controlling this process.

1.2.3 THE ENERGETIC IMPORTANCE OF PROTEIN METABOLISM

The energy costs incurred by a growing animal are the sum of many processes. It would be useful to estimate how much energy expenditure is due to protein metabolism. Metabolizable energy (ME) intake is directed in two ways: to heat output (Ht) and to production such as muscle protein and fat deposition, hair growth and milk.

Heat output depends upon both the size of the animal and the amount it eats. The heat produced when ME is zero and the animal is at rest in a thermoneutral environment and in the postabsorptive

state is known as the basal metabolic rate (BMR). In this situation the animal would be in negative energy balance and lose body mass. When ME intake is sufficient to prevent weight loss but not so large as to cause net production, this is known as maintenance. Upon further increments in ME intake, net production will occur. Increased production is accompanied by increased heat output which increases exponentionally. In other words as ME intake increases so promoting energy retention, the net efficiency with which this occurs declines acceleratingly. However, the higher the production level, the greater the gross efficiency since maintenance energy is always expended whether or not there is any production. Therefore, the higher the production level the less maintenance energy associated with each unit of production. This more than compensates for the extra energy required to deposit increasing units of production.

The problem is to discover what proportion of Ht is due to protein metabolism. There is evidence that protein turnover markedly influences heat output although it is impossible to put a precise figure on it. In the first place it was realised that there was a close association between protein mass and Ht. Pullar and Webster (1974) looked at Ht in obese and lean Zucker rats. They controlled food intake in the two groups and monitered heat loss. It was found that the fatty group which deposited less body protein also lost less heat. When a comparison of Ht was made for lean and fatty groups at similar body weights, again it was consistently shown that Ht was greater in lean rats (Pullar and Webster, 1977). However, as a function of weight of protein, Ht/unit protein was very similar between groups. Furthermore, when the ME requirement for maintenance was compared between species (Webster, 1981) on the

basis of metabolic body weight (Ht/body weight$^{0.75}$) there was considerable variation both between and within species. When Ht was expressed per metabolic protein mass then the difference between fat and thin individuals disappeared. Therefore, it seems that Ht is some function of body protein.

Further work has compared rates of total protein synthesis with total heat loss. Rates of protein synthesis were estimated in lean and fatty rats together with measurements of Ht (Webster, Lobley, Reeds and Pullar, 1978). The correlation between the rate of protein synthesis and Ht was 0.90 which is much higher than the correlation between body protein mass and Ht (0.72) or body weight and Ht (0.59) (Webster et al, 1978). Heat production from individual body organs in the rat has also been measured by implanting catheters and transducers into afferent and efferent blood vessels of organs (Foster and Frydmann, 1978). Heat production for muscle was 20% and liver 12% of total heat production. If these figures are compared with rates of protein synthesis in the rat, it is found that muscle contributes 20% and liver 10% to whole body protein synthesis (Garlick, Burk and Swick, 1976). Thus, a very close correlation between protein synthesis and heat output has been demonstrated. Webster et al (1978) quote that "it appears that protein synthesis and those aspects of metabolism associated with it probably account for about 50% of heat production". Thus, protein metabolism appears to be intimately connected with energy utilization for heat output.

The next question is how much energy directed towards production can be accounted for by lean accretion? Again there is no simple

answer. It will depend upon the proportion of protein in the body and how efficiently protein is deposited. In order to ascertain this, it would be useful to know the energy required to deposit equal amounts of protein and fat (fat being the other major drain on production energy). The energy values of 1g of protein and fat are approximately 23 and 39KJ respectively with the efficiencies of utilization of ME for protein and fat deposition being 45 and 75% respectively (Webster, 1980). Therefore, ME in excess of maintenance to deposit both 1g of protein and 1g of fat is approximately 53KJ (Pullar and Webster, 1977). The body composition of control mice lines selected for appetite was approximately 20% protein, 65% water and 12% fat at 10 weeks (Bishop and Hill, 1985). Mice weighed about 30g at this age. Hence, they contained 6g protein and 3.6g fat. In this example over 60% more ME for production would be directed towards protein deposition than fat accretion. Overall therefore, protein metabolism makes a significant contribution towards the body's energy requirements.

It is worth noting that protein deposited in the body is associated with four times its weight of water. Therefore, lean tissue deposition is more efficient than fat accretion in terms of weight gain/food intake which is a common measure of efficiency.

1.2.4 PROTEIN METABOLISM AND NUTRITION

The mice used in this study were selected on the basis of differences in food intake. Therefore, since protein turnover was investigated in these mice, the established effects of under- and overnutrition upon protein metabolism are reviewed here. Despite

purely manipulative nutritional studies not being directly comparable with alterations in food intake due to selection, it is still important to know how intake may affect protein turnover.

The effects of malnutrition upon different tissues vary according to the susceptibility of a particular organ to privation, the severity of the nutrient deficiency and the period for which nutrients are in short supply. The general response of the whole body to malnutrition is a decrease in the rate of protein turnover. Rats malnourished over a period of ten generations showed decreases in both the rates of whole body protein synthesis and degradation compared with normally fed counterparts (Millward et al, 1974a). Similarly, fasted rats showed a large decrease in the rate of whole body protein synthesis (Millward and Lo, 1977). However, examination of individual tissues does not always reveal the same pattern. For example, in chickens fed a lysine deficient diet, growth rate was decreased in both leg and breast muscle not because the synthesis rate decreased but because the fractional degradation rate was doubled (Maruyama, Sunde and Swick, 1978). This point is also well illustrated by a study that involved feeding a protein-free diet to rats (Garlick, Millward, James and Waterlow, 1975). The gastrocnemius muscle and liver both lost protein but the loss in muscle was due to reductions in synthesis and degradation rates whereas in liver both these parameters increased. Thus, in malnourishment there is no set pattern by which protein is lost from various organs. In other organs examined, intestine, skin and bone, loss of protein was caused by decreased protein synthesis (McNurlan and Garlick, 1979; McNurlan, Tomkins and Garlick, 1979; Preedy, McNurlan and Garlick, 1983).

However, as well as altering with different organs , the mechanism of protein loss can also vary with the type of nutrient deficiency. Comparison of synthesis and degradation rates in skeletal muscle of rats fed either a protein-free diet or starved for three days showed that in the former case, protein loss occurred with only a small decrease in synthesis and a small increase in degradation rates whereas in the latter case a large decrease in muscle protein synthesis and a large increase in catabolism brought about substantial protein loss (Millward, 1970). Therefore, muscle protein turnover appears to be more sensitive to dietary protein/energy deficiency than just protein deficiency.

The period of nutrient deprivation is also important in determining the level and mechanism of protein decrement. For instance, it has been noted in skeletal muscle that acute malnutrition will decrease both protein synthesis and degradation to bring about muscle wasting but then prolonged deficiency accelerated muscle wasting by subsequently increasing catabolism (Millward, Garlick, Nnanyelugo and Waterlow, 1976; Millward and Waterlow, 1978). Hence, it is suggested that skeletal muscle protein is regulated mainly by changes in protein synthesis in all but emergency conditions when an alteration in protein breakdown may be involved.

Apparently, the almost universal reduction in FSR with nutrient deficiency is at first due to a fall in RNA activity followed about 24 hours later by a decrease in RNA concentration which is the main effect (Millward, Garlick, James, Nnanyelugo and Ryatt, 1973). RNA activity does not fall in all cases and may remain unchanged

(McNurlan and Garlick, 1979) or even rise to counteract the main effect of lowered RNA capacity (Preedy et al, 1983).

A notable shift in the source of nitrogen for protein synthesis occurs in skeletal muscle of protein deficient rats. The large decrease in the rate of protein synthesis in this situation can be accounted for entirely by a reduction in protein synthesis from exogenous nitrogen ie from dietary sources whereas the rate of protein synthesis that utilized recycled amino acids remained unchanged and the percentage reutilization of endogenous nitrogen was increased in protein deprived rats (Rikimaru, Yamamoto, Maeda and Inoue, 1980). In addition, different types of muscle appear to be susceptible to privation to a different extent. After one day starvation in rats the rate of protein synthesis had decreased and protein loss had occurred in the white, fast-twitch extensor digitorum longus (EDL) muscle whereas the red, slow-twitch soleus muscle showed no change in either synthesis or breakdown rate (Li and Goldberg, 1976). Over a prolonged period of food deprivation the soleus lost much less weight than the EDL.

Upon refeeding nutrient-deprived animals there are some interesting alterations in protein turnover. The liver, which was shown to be more liable to be affected by nutrient deprivation than many other tissues, accreted protein quickly via increased synthesis and decreased degradation rates (Hill and Malamud, 1974). Skeletal muscle regrowth, however, was achieved more slowly by elevated synthesis and breakdown (Millward et al, 1974b; Funabiki, Watanabe, Nishizawa and Hareyama, 1976). The increase in protein synthesis was due to a higher RNA capacity which raised RNA levels to

pre-deprivation concentrations. RNA activity was also increased but to higher levels than before malnourishment which therefore lead to the achievement of a greater protein synthesis rate than in controls (Millward et al, 1974b).

Hence, protein metabolism is affected by nutrient deprivation according to the length of the deficiency, the nutrients involved and the severity of nutrient withdrawal. Also, the role of individual organs in regulating nutrient supply to the rest of the body is important as well as the type of tissue within a particular organ.

1.2.5 HORMONAL CONTROL OF PROTEIN METABOLISM

It would be impossible to comprehensively discuss the influence of all the major hormones upon protein metabolism and so their main effects will be pointed out with further detail given on the mode of action of exogenously administered anabolic agents. Despite the fact that legislation has banned the use of anabolic agents they will be covered in further detail since these compounds were developed with the intention of maximizing protein deposition in meat animals which is the ultimate aim of this type of genetic study. In this project such an objective would obviously be achieved by a very different method to external hormone administration.

The endocrine system is very highly integrated with the ultimate effects on metabolism being the resultant of the balance of the various hormones. Moreover, protein metabolism is intricately

linked with lipid and carbohydrate metabolism which themselves are influenced by hormones. Thus, it must be emphasized that the resultant level of protein turnover is very much the balance of many factors.

The major hormones that control protein metabolism are: GH, thyroid hormones, corticosteroids, insulin, testosterone and oestrogens. Peptide growth factors are another class of hormones that undoubtedly influence protein turnover but as yet relatively little is known about their role in metabolism and so they have not been included here.

Although GH administration does not always stimulate growth (Muir, Wein, Duquette, Rickes and Cordes, 1975), suppression of the hormones does depress growth (Young, 1980). It has been indicated that inhibition of somatotrophin release-inhibiting factor (SRIF) will increase growth (Spencer and Williamson, 1981; Spencer, Garssen and Hart, 1983). Recently, GH has been shown to stimulate muscle protein synthesis in lambs with a significant increase in muscle growth (Pell, Bates, McAnulty and Laurent, 1988). It also increases RNA polymerase and amino acid transport. Its action may be mediated by somatomedins.

The general action of thyroid hormones is to increase both protein synthesis and degradation with a lack of thyroid hormones resulting in severely retarded growth as illustrated by Snell dwarf mice and children with cretinism. Thyroid hormones are discussed in detail in section 1.3.

Glucocorticoids act catabolically upon protein. There is evidence that loss of protein is caused by both a decrease in protein synthesis and an increase in protein degradation (McGrath and Goldspink, 1978; Kelly and Goldspink, 1982). Since the reduction in the rate of synthesis occurs very rapidly it has been suggested that glucocorticoids decrease the initiation of translation with a rapid decrease in the proportion of polyribosomes (McGrath and Goldspink, 1982).

Insulin is a protein anabolic hormone and it is intimately connected with food intake and nutrition (Garlick, Fern and Preedy, 1983). Streptozotocin-diabetic rats have lower rates of whole body protein synthesis than normal counterparts (Albertse, Garlick and Pain, 1979). It appears that insulin stimulates protein synthesis (Millward, Odedra and Bates, 1983) but inhibits protein degradation (Pain, Albertse and Garlick, 1983). The rate of peptide chain initiation is affected by insulin and without the hormone, polysomal numbers are reduced (Jefferson, Rannels, Munger and Morgan, 1974). This action of insulin on protein synthesis is possibly mediated via prostaglandin $F_{2\alpha}$ (Reeds and Palmer, 1983).

Both testosterone and oestrogens are attributed with having a protein anabolic action and these hormones or their analogues are the most widely used exogenous anabolic agents. Evidence suggests that for maximum growth stimulation, the presence of both androgens and oestrogens is required. Blyth, Cooper, Roobol and Ratin (1972) showed that the activation of the microsomal membrane towards polysome binding requires both an oestrogen and an androgen. Therefore, female animals respond well to androgenic agents, males

to oestrogenic agents and castrates to a combination of these (Best, 1972; Heitzman and Chan, 1974; Chan, Heitzman and Kitchenham, 1975; Galbraith and Watson, 1978). The mode of action of oestrogenic agents is not very clear but it is thought that they may elicit an effect by increasing endogenous insulin and growth hormone levels (Preston, 1975; Trenkle, 1976) which is consistent with elevated protein synthesis and growth rate. On the other hand, androgenic anabolic agents do not appear to have very much influence upon endogenous hormone levels. The only major hormones affected by trenbolone acetate (TBA), an artificial androgen, were thyroxine which was reduced in the plasma of treated steers (Heitzman, Chan and Hart, 1977) and glucocorticoids which were reduced in female lambs (Thomas and Rodway, 1982). Interestingly, TBA has been shown to decrease both the rates of protein synthesis and protein degradation (Vernon and Buttery, 1976) which is consistent with decreased thyroid hormone and glucocorticoid levels whereas, testosterone increased the rate of protein synthesis (Martinez, Buttery and Pearson, 1984). Therefore, these two anabolic agents must have different modes of action. Current theory suggests that anabolic androgens may act upon muscle in a number of ways. Firstly, they may bind with a muscle receptor and exert a direct effect upon muscle protein metabolism. The interaction of TBA with cytosolic testosterone receptors has been observed in sheep muscle (Sinnett-Smith, Palmer and Buttery, 1987). It is also postulated that anabolic agents may exert a growth promoting effect by displacing glucocorticoids from their cytoplasmic receptors so reducing their catabolic effects (Mayer and Rosen, 1975). In addition, circulating glucocorticoid concentrations may be reduced. However, it is difficult to come to any firm conclusions as to their

mechanism of action.

Of the hormones known to influence protein metabolism the thyroid hormones are particularly interesting in the context of this work. They are therefore reviewed in more detail in the following section (section 1.3).

This section has outlined the major environmental factors that affect protein turnover and will enable the effects of gentoype upon protein metabolism to be compared with those that occur with more conventional manipulations such as nutrition.

1.3 THYROID HORMONES

The initial aim of this study was to investigate the effect of selection for appetite upon protein turnover. After the first experiment, since protein turnover is affected by many hormones it was decided to try and link differences in protein metabolism with a hormonal factor. This would also have the advantage of being much easier and cheaper to measure as a selection parameter than protein turnover. Thyroid hormones were chosen to be investigated because they have been shown to affect protein turnover, are connected with appetite and are genetically controlled.

1.3.1 DISCOVERY, BIOSYNTHESIS AND CIRCULATION

The thyroid hormones are iodinated derivatives of tyrosine that are synthesized in the thyroid gland situated at the base of the neck. Although thyroxine (tetraiodothyronine, T_4) was discovered sixty years ago (Harington and Barger, 1927), tri-iodothyronine (T_3) was not recognized until 1952 (Gross and Pitt-Rivers, 1952; Roche, Michel and Lissitzky, 1952).

There are three main stages in thyroid hormone synthesis. The first is transfer of iodide into the thyroid. The body's iodine comes from the diet. It is actively accumulated in the thyroid at levels up to twenty times the concentration in the blood. This process requires both ATP and glucose and is closely associated with the Na^+K^+ pump. The second stage is transformation of iodide to an oxidized form of iodine and iodination of tyrosyl residues. The

thyroid gland consists of lobules which each contain 20-40 follicles bound by connective tissue. The follicle walls are made up of a single layer of secretory epithelial cells which surround a secretory lumen. It is thought tyrosyl residue iodination occurs at the interface of the secretory cell and follicular lumen although some intracellular iodination may occur. The final stage in thyroid hormone synthesis is coupling of iodotyrosines. T_4 is made from coupling two di-iodotyrosine residues (see Fig 1.3.1.1a) and is the main secretory product. T_3 is formed from one di-iodotyrosine and one mono-iodotyrosine residue (see Fig 1.3.1.1b). T_3 can also be produced by deiodination of T_4 since the thyroid gland contains some iodotyrosinase deiodinases. The deiodinating activity may vary according to the body's iodine status, ie when iodine is deficient, T_3 synthesis is increased. The iodothyronines thus formed are contained within the follicular lumen in colloid form bound to thyroglobulin. Before secretion into the bloodstream the hormones must be released from the thyroglobulin.

Upon entry into the bloodstream the thyroid hormones are bound to other proteins. Thyroxine-binding globulin (TBG) is an α-glycoprotein which has a low affinity for T_3 but a high affinity for T_4 albeit a low capacity. The other major circulatory protein to which thyroid hormones bind is thyroxine-binding pre-albumin (TBPA) which also possesses a high affinity for T_4. Binding is reversible and probably governed by electrostatic forces. A little T_4 and T_3 is present in the circulation in the free state and this is important since only the free hormones diffuse into tissues and are responsible for biological activity. The proportion of circulating free hormone can vary due to changes in TBG levels

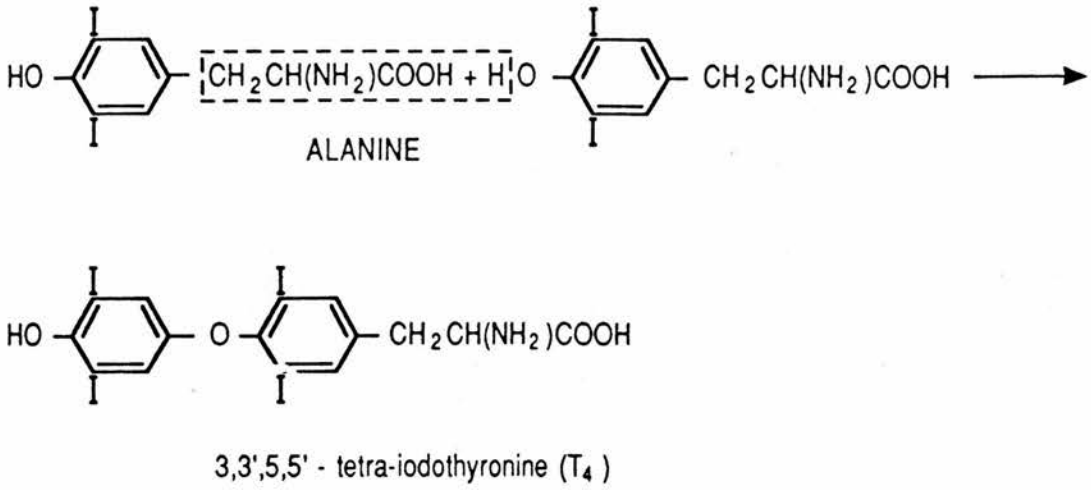


Figure 1.3.1.1a Thyroxine formation by coupling two di-iodotyrosine residues.

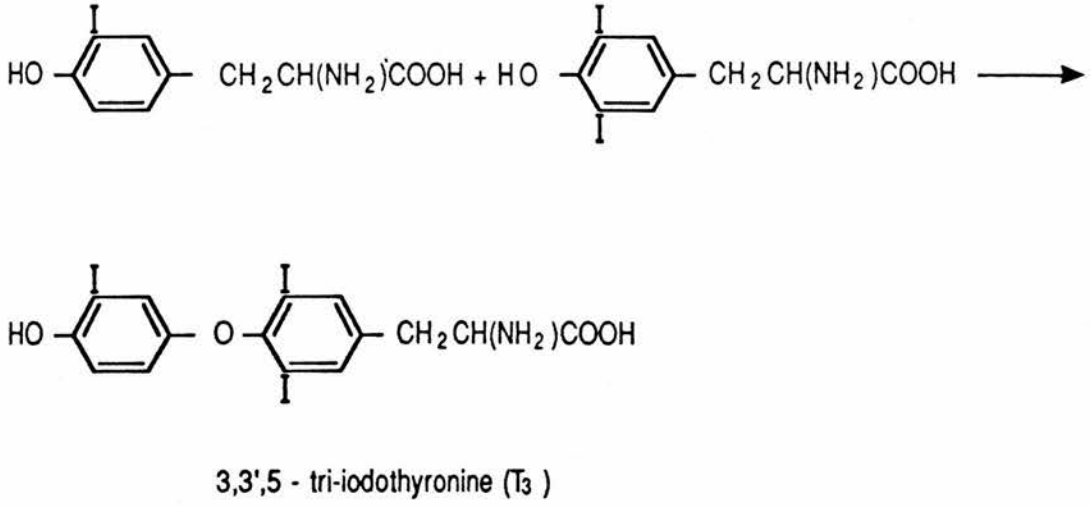


Figure 1.3.1.1b Formation of tri-iodothyronine from mono-iodotyrosine and di-iodotyrosine.

according to conditions. It has been shown that chronic underfeeding is associated with supra-normal TBG levels and hence a low percentage of free T_3 (Glass, Young and Anderson, 1986).

Even though T_4 forms the greatest proportion of thyroid hormone secreted from the thyroid gland (90-95%; Chopra, Solomon, Chopra, Wu, Fisher and Nakamura, 1978) it is thought that T_3 is the metabolically active form. Much T_4 is peripherally processed to T_3 and so T_4 could be considered to be a prohormone. Most peripheral T_4 5' monodeiodination is believed to occur in the liver and kidneys (Hillier, 1972; Chiraseveenuprapund, Buergi, Goswami and Rosenberg, 1975). Approximately 80% of circulating T_3 in man is produced this way. As well as peripheral T_3 production by deiodination, a parallel process produces reverse T_3 (rT_3) which is metabolically inactive. The balance of T_3 and rT_3 production varies with the body's metabolic state and adapts accordingly; for instance, during starvation the T_4 to T_3 conversion pathway is inhibited while the T_4 to rT_3 pathway is fully operational (Chopra et al, 1978). Further deiodination of rT_3 to di-iodothyronines and mono-iodothryonines also occurs (Chopra et al, 1978).

1.3.2 CONTROL OF CIRCULATING THYROID HORMONE CONCENTRATIONS AND PERIPHERAL METABOLISM

The hypothalamus secretes thyrotropin releasing factor (TRF) (also called thyroid stimulating hormone-releasing hormone, TSH-RH). TRF is a weakly basic tripeptide (Fig 1.3.2.1): L-pyroGlu-L-His-L-Pro-amide, that stimulates thyroid stimulating hormone (TSH, thyrotropin) secretion from the anterior pituitary.

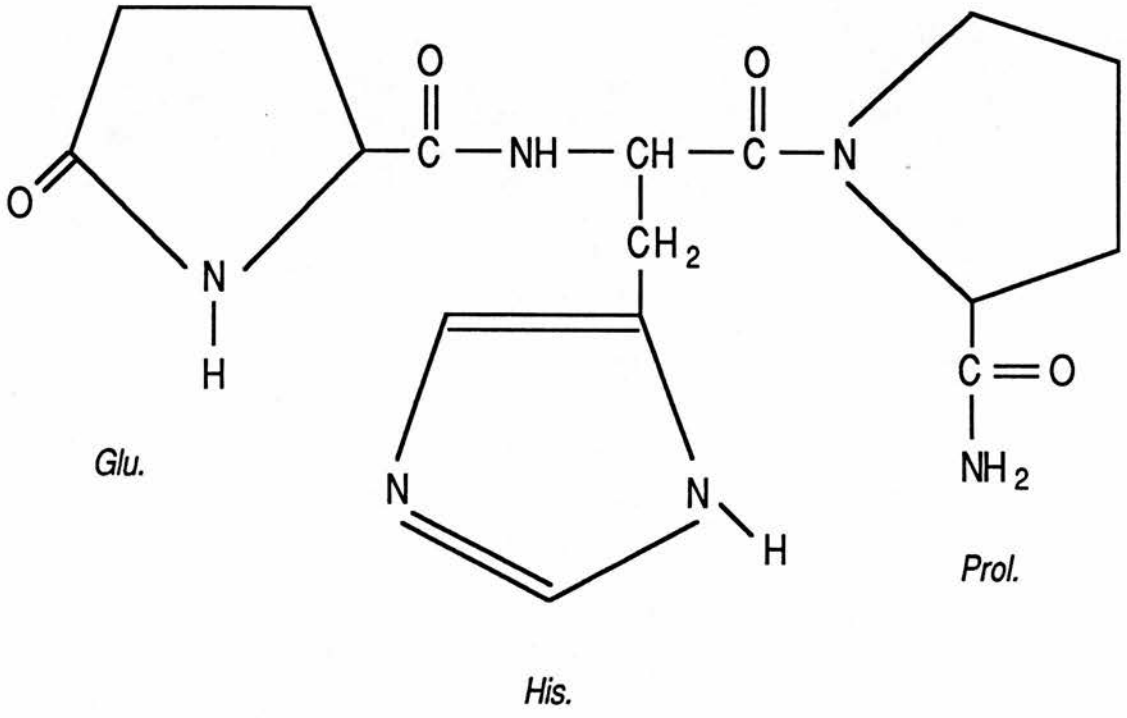


Figure 1.3.2.1 The structure of Thyrotropin Releasing Factor (TRF)

In turn, TSH which is a glycoprotein produced from the basophils in the pars distalis, promotes thyroid growth and liberation of thyroid hormones from the follicular colloid.

Although TSH regulates the activity of the thyroid gland, circulating thyroid hormones feedback and exert fine control upon this central modulation of their production. In fact, thyroid hormones override higher centre control of thyroid hormone secretion. There appears to be a better inverse relationship between TSH and T_4 rather than T_3 (Larsen, Silva and Kaplan, 1981) which is consistent with a primary role for T_4 in the regulation of TSH production. Thus, T_4, the prohormone is the first level of feedback. However, it has been established that only T_3 (free) will bind to anterior pituitary nuclear thyroid hormone receptors and that much intracellular T_4 deiodination occurs in this tissue. Approximately 50% of anterior pituitary intracellular T_3 can be attributed to intracellular T_4 deiodination and 50% is derived from the plasma. In other tissues such as liver, heart, skeletal muscle and kidney most intracellular T_3 originates from plasma. Thus, changing plasma T_4 concentrations will considerably affect intracellular T_3 levels in anterior pituitary but not in peripheral tissues. Therefore, for example, in a situation of thyroid hormone imbalance such as mild hypothyroidism, firstly the proportions of T_3 and T_4 secreted from the thyroid gland increase and decrease respectively. Hence, plasma T_3 levels are maintained and no metabolic effects of the imbalance can be detected by peripheral tissues. However, a low T_4 concentration is detected in the anterior pituitary because T_3 production from intracellular T_4 to T_3 conversion is diminished and consequently TSH production and release

is stimulated. Therefore, thyroid hormone levels are restored. On the other hand, if plasma T_3 levels were altered with accompanying serious implications and effects in peripheral tissues, the anterior pituitary would again be stimulated to alter TSH production and release. Since T_3 is ten times more potent than T_4, then a much smaller change in T_3 levels would restore the balance. Thus, using a prohormone as the first feedback level against imbalance provides the opportunity of correcting inequalities without overt clinical symptoms but an even quicker response may be elicited if a more serious situation arises with an upset in the level of the active hormone itself. It is clear from this that T_3 and TRF are mutual antagonists with respect to thyroid hormone synthesis and release. The active hormone, T_3, overrides the influence that TRF exerts upon the pituitary.

Thyroid hormone action is initiated at the nuclear level by direct binding to specific receptors (Oppenheimer, Schwartz, Surks, Koerner and Dillman, 1976). The interaction of receptors with chromatin components is an important aspect of the action of thyroid hormones and evidence suggests that the nuclear hormone receptor complex acts by modulation of specific mRNA molecules at transcription or at a posttranscriptional event (Samuels, Perlman, Raaka and Stanley, 1982). There are no cytosolic receptors but brain synaptosomal T_3 binding sites exist (Imura, Tanaka, Mashio, Ishii, Inada and Nishikawa, 1984). The biological action of T_3 at synaptosomal sites is still unknown.

Nuclear receptors have approximately a 5 hour half life (Raaka and Samuels, 1981) and so are a dynamic population. A variety of

physiological and pharmacological factors influence the thyroid hormone receptor population. Association of the hormone with the receptor decreases receptor numbers as does starvation or a reduction in carbohydrate intake. Administration of pharmacological doses of glucagon has the same effect (Samuels et al, 1982). Therefore, alteration of the receptor numbers is a vital method of modulating thyroid hormone action as well as changing the levels of circulating hormones themselves.

1.3.3 METABOLIC EFFECTS OF THYROID HORMONES

Thyroid hormones have two main types of action: calorigenic effects and growth promoting/developmental effects. The calorigenic action is often involved in changes in thermogenic responses and in responses to changes in nutrition. This includes alterations in glucose metabolism, oxygen consumption, futile and substrate cycling. The growth action is concerned with metamorphic development in amphibians and in mammals with changing rates of protein synthesis and degradation and with skeletal growth.

1.3.3.1 Calorigenic Effect

It is well documented that central core temperature is elevated in hyperthyroidism and reduced in hypothyroidism (Himms-Hagen, 1976; Sestoft, 1980). Moreover, acute cold exposure in rats increased serum T_3 and T_4 concentrations within two hours (Hefco, Krulich, Illner and Larsen, 1975) and chronic cold adaptation also raised thyroid hormones levels (Pueria, Abelenda and Fraile, 1984). In addition, acute heat stress has been shown to reduce circulating T_3

to half baseline levels with a concomitant increase in rT_3 (Konits, Hamilton, Pruce, Whitacre and Van Echo, 1984). From these and many other studies a permissive role for thyroid hormones in increased thermogenesis during cold exposure has been postulated.

The other major environmental factor affecting thyroid hormone metabolism is nutrition. Overnutrition increases, and starvation decreases, thyroid metabolism (Ingenbleek and Beckers, 1975; Millward, Holliday, Bates, Dalal, Cox and Heard, 1979; Ortiz-Caro, Gonzalez and Jolin, 1984). However, the response is very dependent upon the composition of the diet. After feeding obese patients with isocalorific diets consisting of either 100% carbohydrate or 20% protein and 80% fat or 20% protein, 25% carbohydrate and 55% fat (Spaulding, Chopra, Sherwin and Lyall, 1976) it was suggested that dietary carbohydrate was the major factor controlling T_3 concentrations. T_3 levels fell only on the carbohydrate-free diet and the decrease was equivalent to that observed during starvation. Later studies modified this theory slightly and lead to the suggestion that T_3 production is sensitive to the change in carbohydrate intake rather than the absolute level (Azizi, 1978; Jung, Shetty, Barrand, Callingham and James, 1978). Carbohydrate intake affects nuclear T_3 receptors as well as plasma T_3 concentrations (section 1.3.2).

The calorigenic action of thyroid hormones induced by environmental temperature and nutritional changes is characterized by several metabolic responses. These include alterations in glucose metabolism, oxygen consumption, heart rate and substrate recycling as well as adaptive responses in different body tissues to

facilitate these actions.

There are three ways in which thyroid hormones induce changes in glucose metabolism. Carbohydrate uptake from the gut is stimulated by thyroid hormones (Holdsworth and Besser, 1968; Marechaud, Rambaud and Matuchansky, 1980) and also there is enhanced endogenous glucose production by liver and kidney (Okajima and Ui, 1979; Huang and Lardy, 1981; Muller, Paschen and Seitz, 1983). These two types of response to thyroid hormones increase the provision of glucose. Thyroid hormones also impose an increased energy demand on the body which is the third effect of thyroid hormones on glucose metabolism. For example, hyperthyroidism increases the rate of glucose uptake and oxidation by many tissues such as heart, adipocytes and skeletal muscle (Burns and Reddy, 1975; Czech, Malbon, Kerman, Gitomer and Pilch; 1980).

T_3 has been demonstrated to directly stimulate oxygen consumption and ATP synthesis in a dose-dependent manner in various tissues (Sterling, 1979; Muller and Seitz, 1980). Consequently, the respiration rate and heart rate are also raised (Sestoft, 1980). In addition, energy consuming reactions are stimulated by T_3. Various reactions in intermediary metabolism have been implicated as contributing towards the elevated heat production observed during hyperthyroidism. For instance, the energy expenditure of the Na^+K^+-ATPase enzyme (sodium pump enzyme) was significantly increased in the hyperthyroid state and decreased in hypothyroidism (Lin, Vander Tuig, Romsos, Akera and Leveille, 1979; Smith and Edelman, 1979). Thyroid hormones have also been demonstrated to increase total body energy consumption by a series of cyclic reactions known

as "futile" cycles. Both intra- and interorgan futile cycling occurs. Intraorgan substrate recycling involves cells containing a pair of catabolic and anabolic enzymes catalyzing opposing reactions eg hexokinase and glucose-6-phosphatase, phosphofructokinase and fructose bisphosphatase in glucose metabolism. Under normal physiological conditions these reactions are irreversible but the activity of thyroid hormones and catecholamines will stimulate cycling (Rognstad, 1977; Okajima and Ui, 1979; Huang and Lardy, 1981). For each substrate recycled 1 ATP is lost. Interorgan futile cycling includes glucose catabolism via glycolysis in muscle cells to lactate and glucose regeneration via hepatic gluconeogenesis (Cori cycle). Such substrate recycling releases the energy from 4 ATP molecules per substrate molecule recycled. Huang and Lardy (1981) observed by a series of labelled glucose experiments that the Cori cycle and Felig's cycle (glucose → alanine → glucose) were increased by as much as 33-100% in hyperthroidism and decreased by 22-30% in hypothyroid rats. At the intraorgan level three fold increases in futile cycle rates were observed during hyperthyroidism and half normal rates in thyroidectomized rats. Interorgan futile cycles also include concomitant elevation of lipid and protein synthesis and breakdown rates. Protein turnover will be discussed in detail in the next section (1.3.3.2). Futile cycling accounts for a significant part of the thermogenesis through increased metabolic rate due to thyroid hormones and they also explain why thyroid hormones stimulate oxygen consumption.

1.3.3.2 Growth Promoting Effect of Thyroid Hormones

Although thyroid hormones are attributed with an anabolic action on protein metabolism this action is very dependent upon thyroid hormone dosage and the individual tissue concerned. Many studies have demonstrated that thyroid deficiency or malnourishment will decrease the rate of whole body growth. This is achieved by reductions in both the rates of protein synthesis and degradation whereas these parameters are increased by the addition of thyroid hormones to improve the body's nitrogen balance (Millward et al, 1979; Brown and Millward, 1983). However, thyrotoxicity can occur if thyroid hormone levels are excessively high with accompanying loss in body mass (Rupp, Paschkis and Cantarow, 1949; Carter, Benjamin and Faas, 1980; Angeras and Hasselgren, 1987).

Changes in rates of protein growth with alterations in thyroid hormone status are not uniform throughout the whole body. Much evidence indicates that skeletal muscle and liver growth is affected in the same way as whole body growth by different thyroid states. However, the growth rate of cardiac muscle is only reduced slightly during hypothyroidism. Also, protein loss due to thyrotoxicosis does not occur at all in this tissue (Carter et al, 1980) although thyroid-induced cardiac hypertrophy has been observed (Sanford, Griffin and Wildenthal, 1978). Thus, different tissues appear to have a different responsiveness to thyroid hormones. Hypothyroidism causes decreases in both protein synthesis and degradation rates in skeletal muscle and liver (Brown, Bates, Holliday and Millward, 1981; Brown and Millward, 1983; Martynenko and Kornyushenko, 1984) whereas hyperthyroidism has the opposite

effect causing an increase in synthesis and degradation in both these tissues (Hasselgren, Adlerberth, Angeras and Stenstrom, 1984; Martynenko and Kornyushenko, 1984; Gallo, Voci, Scwarze and Fugassa, 1987). Since thyroidectomy reduces growth rate, then the fall in synthesis rate is greater than that in breakdown rate and vice versa in hyperthyroidism. However, as far as heart tissue is concerned, alterations in the rate of protein synthesis appear to be the only change. During T_4-induced cardiac hypertrophy, instead of increases in the rates of synthesis and degradation, a large increase in synthesis occurs with no change in degradation (Carter, Benjamin and Faas, 1980; Carter, Benjamin and Faas, 1982). Upon regression of hypertrophy the rate of protein synthesis decreases with no change in the rate of breakdown (Sanford, Griffin and Wildenthal, 1978). This contrasts markedly with reactions in skeletal muscle and liver.

Thyroid hormone-induced changes in protein synthesis are caused in various ways. Thyroidectomy has been shown to reduce skeletal muscle and liver RNA concentration and activity (Flaim, Li and Jefferson, 1978; Brown et al, 1981; Peavy, Taylor and Jefferson, 1981; Brown, Van Buenen and Millward, 1983) indicating that thyroid hormones control the rate of protein synthesis by stimulation of RNA production. However, thyroid hormones also stimulate peptide synthesis by a direct action at polysome level (Mathews, Oronsky and Haschemeyer, 1973; Carter, Faas and Wynn, 1975) which is not attributable to an increased RNA capacity. A further study indicated that T_4 encouraged peptide chain elongation which may indicate a reaction at the level of aminoacyl-tRNA or peptide bond synthesis (Carter, Faas and Wynn, 1976). Hence, thyroid hormones

appear to stimulate protein synthesis by at least two different methods.

Furthermore, it seems that synthesis rates are influenced by thyroidectomy more easily in some muscles/tissue fractions than others. Evidence points towards fast-twitch glycolytic muscles such as plantaris and gastrocnemius muscles having a faster reduction in synthesis rates after thyroidectomy than slow muscles (King and King, 1973; Brown and Millward, 1983). Conversely, slow-twitch muscles (eg soleus) showed elevated degradation rates much quicker than fast muscles after thyrotoxic T_3 administration (Angeras and Hasselgren, 1985). The components of liver protein are also subject to disparity in their reaction to thyroid hormones. Upon thyroidectomy total liver secretory protein synthesis was reduced by 50% but there was only a 20% decrease in intracellular protein synthesis (Peavy et al, 1980). When thyroid hormone levels were restored, the synthesis rate of intracellular proteins increased by 115% whilst that of secretory proteins only increased by approximately 60%.

Alterations in liver and skeletal muscle degradation rates due to thyroid hormones have been correlated with changes in both the number and activity of lysosomal enzymes such as cathepsins B and D (De Martino and Goldberg, 1978; Goldberg, Tischler, De Martino and Griffin, 1980; Krupp, Starling, Golstein and Neve, 1984). However, a recent study has indicated that T_4 may regulate skeletal muscle protein degradation by enhancing Ca^{2+}-stimulated non-lysosomal degradation whilst inhibiting lysosomal/autophagic proteolysis by a Ca^{2+}-dependent mechanism (Zeman, Bernstein, Ludemann and Etlinger,

1986). Therefore, despite evidence that T_4 may increase lysosomal proteinases it must be remembered that enzyme levels do not reflect fluxes though pathways. Hence, the mechanism by which thyroid hormones stimulate protein degradation has not been positively identified.

1.3.4 THYROID HORMONES: GENETIC VARIATION

It was established many years ago that thyroid activity and control is influenced genetically. Strain differences were found in characteristics such as morphological appearance of the thyroid gland (Jacobs, 1958; Wykes, Christian and Andrews, 1958; Silverstein, Sokoloff, Mickelsen and Jay, 1960), $^{[131]}I$ uptake (Wykes et al, 1958) and response to goitrogens (Wilson, 1952). More recently mice with mutations for a single gene have reinforced the idea of genetic influence upon thyroid activity. Upon T_4 treatment both Snell dwarf (dw/dw) and Ames dwarf (df/df) mice grow more rapidly to within the normal range (Bartke, 1965; Holder and Wallis, 1976; Holder and Wallis, 1977; Van Buul Offers, Smeets and Van den Brande, 1984) suggesting that in both these dwarf strains a direct effect of the gene on the thyroid/thyroid receptors/$T_4 \rightarrow T_3$ conversion may be involved. It has also been established that obese (ob/ob) mice are functionally hypothyroid (Lin et al,1979). There is evidence that hepatic and renal peripheral deiodination is impaired in this strain (Hillgartner and Romsos, 1985). This may result in decreased T_3 availability to thermogenic target areas. Hence, energy dissipation must occur elsewhere, namely as fat deposition which acts as an energy sink. Therefore, there is ample evidence that there is genetic control of thyroid hormone activity.

Since thyroid hormones have a profound influence upon growth and development it is interesting to see if strain differences in growth rate can be related to diversity in thyroid hormone metabolism. Thyroid hormones may make some contribution to these differences although they would not be expected to be the sole determinant of growth rate differences between strains of animals.

When three strains of broiler chickens were compared for plasma T_3 concentrations, the fastest growing strain also had the highest plasma T_3 concentrations (Stewart and Washburn, 1983). Similarly, a positive correlation was shown between body weight and thyroid activity in mice selected for large and small size (Synenki, Eisen, Matrone and Robison, 1972) although only a small portion of body weight variation could be accounted for by the difference in thyroid activity. In addition, a high thyroid activity was correlated with the fastest growth rate in young bulls (Sorensen, Kruse and Andersen, 1981). When mice were selectively bred for $^{[131]}I$ uptake into the thyroid gland a four fold divergence in this parameter was evident after ten generations (Chai, 1970). Moreover, the mice with high $^{[131]}I$ uptake had a higher BMR, a faster growth rate and matured earlier than mice with a low $^{[131]}I$ uptake. In an experiment where the link between T_4 and lactational performance was being examined, a positive correlation was also found between plasma T_4 concentration and total litter weight at twelve days and in individual weight at three, six and nine weeks (Tilakaratne, Hill and Land, 1981). However, in this experiment, all the variation in litter weight could be accounted for by differences in maternal milk production.

On the other hand, in a number of studies including strains of rat (Kuhn et al, 1983), mice (Edwards, 1962; Esber et al, 1974), pigs (Kasser et al, 1981) and humans (Kvetny, 1985) no correlation between thyroid hormones and body weight was apparent. Therefore, there is some evidence that thyroid hormones may influence growth rate differences between strains of animals but they are not involved in all cases.

This section has outlined how thyroid hormones are synthesized, metabolized and influenced by environmental factors with their subsequent effects on the body's metabolism. They are involved in the regulation of protein turnover, influenced by nutrition and are also under genetical control. This makes them good candidates for examination in relation to mice selected for appetite and the control of differences in protein turnover in these animals.

CHAPTER TWO

GENERAL METHODS

2.1 EXPERIMENTAL INTRODUCTION

The initial approach in this work was to quantify fractional rates of protein synthesis and degradation in various tissues of the selected lines to see if they differed. The rate of protein synthesis was measured directly by injection of a very large dose of labelled phenylalanine (Garlick, McNurlan and Preedy, 1980). The principle behind this method is that injection of a flooding dose of a radioactive amino acid will saturate all the free amino acid compartments. Therefore, measurement of the specific activity of the precursor amino acid pool is simplified because the specific activity of the various free pools which can be measured easily should be close to that of the true precursor pool, the aminoacyl-tRNA, which is very difficult to measure. Additionally, only a slow and linear fall in the free amino acid specific activity will occur which avoids using large numbers of animals to determine a time course.

Protein degradation was measured indirectly by finding the difference between synthesis and growth rates. Growth rates were found by taking mice at two different ages and protein contents of the various tissues determined. Assuming growth to be linear over the period between the two measurements, the fractional rate of protein growth can be determined.

Ideally the rate of protein degradation would have been measured directly but no suitable method is available for _in vivo_ studies in mice. The popular method of measuring N^{τ}-methylhistidine excretion is unsuitable because this amino acid is metabolized in mice

(Murray, Neild, Jones, Galbraith and Tomas, 1985; Harris, Rucklidge, McDiarmid and Milne, 1986).

Upon completion of this study which indicated that differences in rates of protein turnover may be an important factor in causing the diversity of the selected mice strains, a further investigation to try and link these differences to hormonal factors was instigated. Protein turnover is under the influence of many hormones (section 1.2.5) and so it was logical to go into more detail to see if any of the differences could be accounted for by hormonal differences. Measurement of hormone concentrations is also much cheaper and simpler than measuring protein turnover and so finding a hormonal connection would be better as far as a selection criterion is concerned. Thyroid hormones were singled out for investigation because they are known to influence protein metabolism and they are concerned with appetite control which is the original parameter of selection. Evidence that thyroid hormones have accounted for some growth differences between strains of animals is also available (section 1.3.4). First the concentrations of thyroid hormones were determined in the plasma to see if they were different between strains. Results from this study warranted a further experiment to investigate whether differences in protein turnover and thyroid hormone concentrations were connected. This was approached by reducing thyroid hormone levels to zero and then replacing the same amount of thyroid hormone to each line. The response in protein turnover was determined.

The rest of this chapter describes methods and procedures required to perform the studies outlined above and gives the background of

the appetite selected mice.

2.2 ANIMALS

2.2.1 Management and Stock

Mice were given free access to food (Beta Diets Rat and Mouse No 1 Expanded Maintenance Diet, 14.8% crude protein, 13.1 kJ/g metabolizable energy) and water. They were housed at 20°C on a natural daylength cycle.

The mouse selection lines were established by crossing two inbred lines (Sharp et al, 1984). The F_1 was crossed with an outbred strain whose offspring were allowed to mate randomly for a further generation. The next generation was designated as generation 0. Three contemporary lines were selected upwards, downwards and there was an unselected control. For each of these selection lines three replicates were maintained. This study was initiated at generation 21 of selection. At generation 23 another replicate, replicate 6, was formed by crossing stock from the three original replicates. Also, at this stage selection was relaxed in replicates 1, 2 and 3 due to inbreeding and health problems but selection continued in replicate 6 throughout the period of study. The generation of selection reached in individual trials is indicated in the appropriate chapters. At generation 0 sixteen full-sibling families were established for each replicate of each selection treatment. Sixteen pair matings were taken for each replicate until generation 8 when they were reduced to eight pair matings. Selection was within litters. To spread the work load, matings for the different

replicates were set up at approximately 4 week intervals but lines within a given replicate were kept as contemporaries. Litters were adjusted to between six and twelve young at birth.

2.2.2 Selection Procedure

The selection criterion was 4-6 week food intake with a correction for any differences in 4 week weight since obviously any differences in initial weight will affect subsequent intake. The adjustment for differences in 4 week weight was by within-family, within-sex phenotypic regression of food intake on 4 week weight. These regression coefficients were calculated to be 1.65 and 2.21g food/ g body weight for females and males respectively. Mean 4 week weights were found to be 16.1 and 17.8g (females and males respectively). Therefore, the adjusted food intake was calculated as:

Males = 4-6 week food intake + 2.21(17.8 - 4 week weight).

Females = 4-6 week food intake + 1.65(16.1 - 4 week weight).

One mouse of each sex per litter was selected for the following generation.

2.2.3 Responses: direct and correlated

After 11 generations of selection, figure 2.2.1 shows that adjusted food intake was altered by selection. The H lines had increased their adjusted food intake by approximately 8% and the L lines decreased it by about 8.6%. This represents a total divergence of 1.9 phenotypic standard deviations. Food intake *per se* and food intake scaled to both body weight and to metabolic body weight also followed a similar pattern.

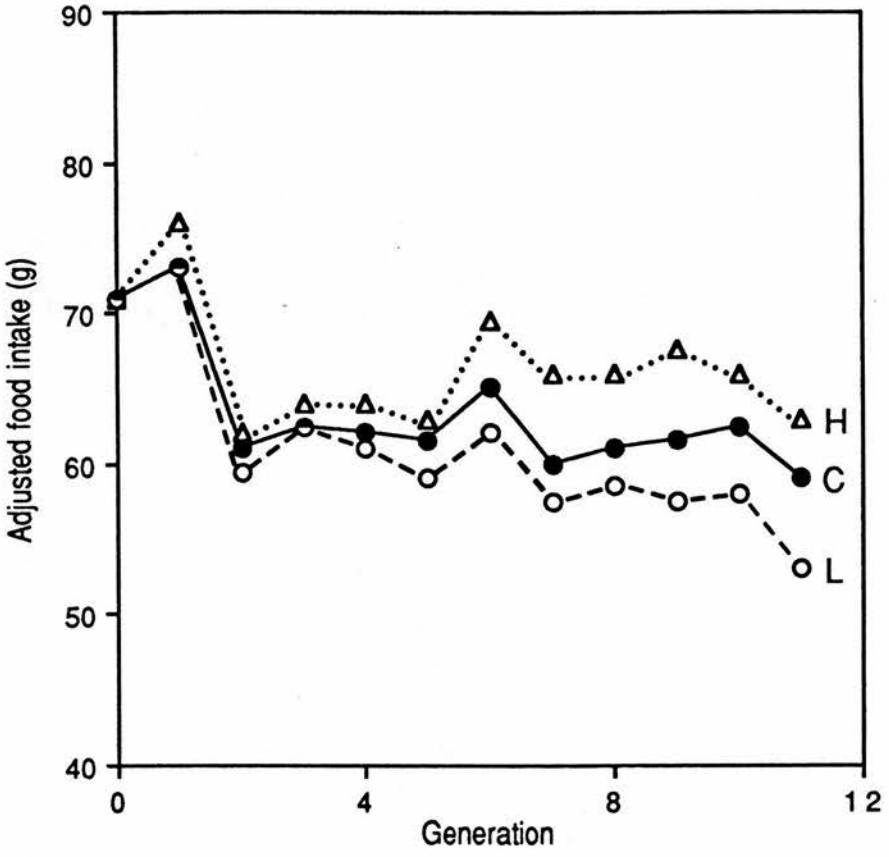


Figure 2.2.1 Adjusted food intake for the mean of replicates 1, 2 and 3 in mice selected for appetite.

(Sharp, Hill and Robertson. 1984)

After 14 generations the growth curve (figure 2.2.2) clearly shows that until 4 weeks of age all lines have similar body weights but thereafter they diverge. Carcass composition is also altered. H lines have less fat but more water and protein. It has been suggested that the changes in body composition are caused by the restriction in 4 week body weight since these differences are apparent at this age but there are no subsequent changes in body composition.

Whole body maintenance requirements have also been investigated and when scaled to both metabolic body weight and to metabolic lean weight it was found that the H line had larger maintenance requirements than the L line (Bishop and Hill, 1985).

2.3 PROTEIN CONCENTRATION

Protein concentrations were determined using the method of Lowry, Rosebrough, Farr and Randall (1951) as modified by Wang and Smith (1975). To duplicate 0.2ml aliquots of the tissue extract, dissolved in 0.3M sodium hydroxide containing 0.1% Triton X-100, 1.0ml of alkaline copper reagent was added (0.025% (w/v) copper-EDTA, 2% (w/v) sodium carbonate in 0.1M sodium hydroxide) and the mixture shaken. After 15 minutes at room temperature, 1.0ml of 10% sodium dodecyl sulphate (w/v) was added, the tubes shaken and 0.1ml of Folin reagent (Folin- Ciocalteau's phenol reagent 1:1 with water) added and the mixture shaken again. After 30 minutes incubation at room temperature the absorbance of the mixture was measured at 750nm using a Pye Unicam PU 8610 spectrophotometer. The same procedure was performed on duplicate sets of bovine serum

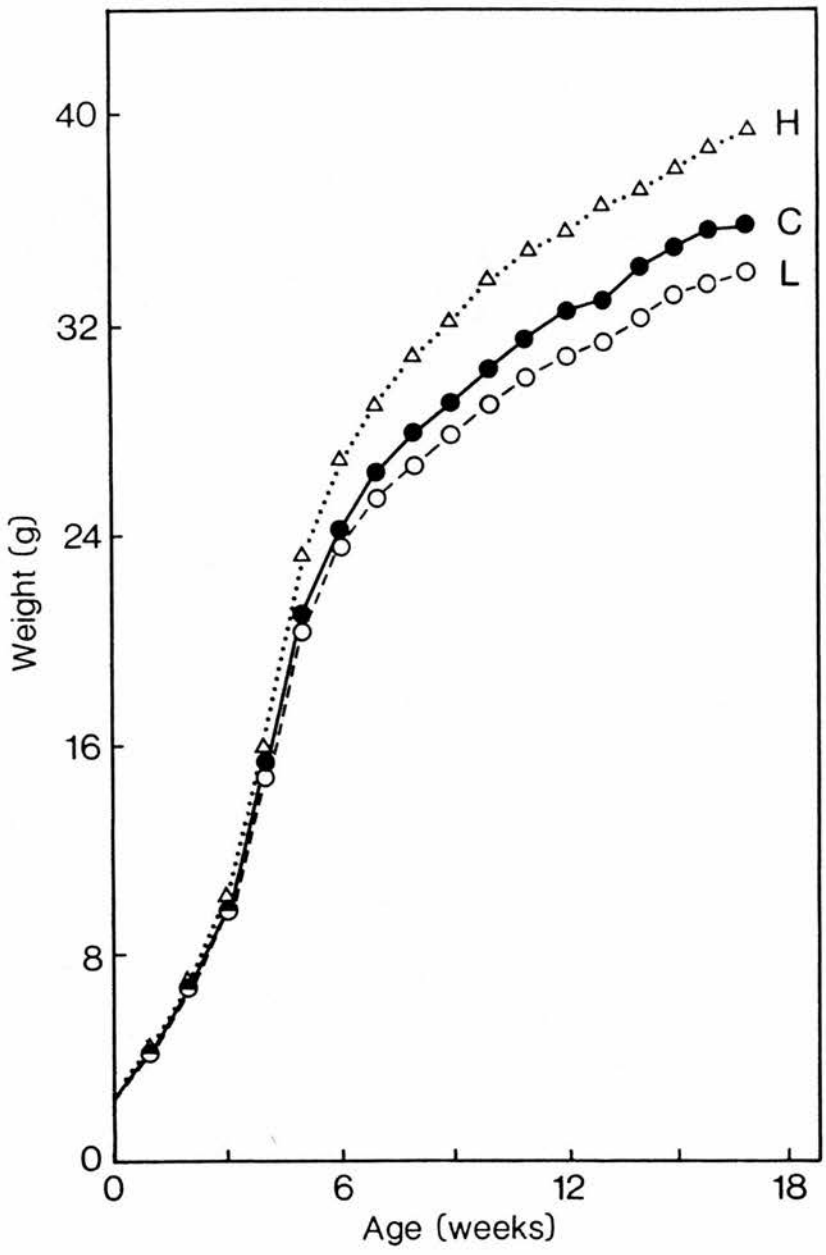


Figure 2.2.2 Growth curves of appetite selected mice.
Δ high and O low appetite, ● control.

(from Bishop and Hill, 1985)

albumin at concentrations of 0, 10, 20, 40, 60, 80 and 100 μg/tube to obtain a standard curve. The absorbance range of the standard curve was 0 to 0.6 and the absorbance of the samples varied from 0.06 to 0.6.

2.4 DNA DETERMINATIONS

All DNA determinations were performed using the ethidium bromide method of Karsten and Wollenberger (1972) as modified by Boer (1975) for small quantities of tissue. Tissue samples were homogenized in 0.5ml of ice-cold pronase (0.1mg/ml; Protease, Streptomyces griseus 110,000 PUK/g; Calbiochem) in phosphate buffered saline/EDTA (pH 7.5). After addition of 0.5ml ribonuclease A (100μg/ml in PBS; bovine pancreas type 1-A; Sigma) and shaking, the mixture was incubated at 37°C for 30 minutes. Then, 3.5mls of PBS/EDTA were added and the fluorescence of the mixture measured at 366nm excitation and 590nm emission wavelengths on a Perkin-Elmer LS-2 fluorimeter. The fluorescence measurement was repeated after addition of 0.5ml ethidium bromide (25μg/ml). A standard curve was obtained by following the same procedure on duplicate sets of DNA (calf thymus type 1 sodium salt, highly polymerized; Sigma) at concentrations 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 μg/ml and the difference between fluorescence before and after ethidium bromide addition plotted against concentration. Typical ranges for standard curves were 0 to 250 and for unknowns were 50 to 200.

2.5 RNA DETERMINATIONS

All RNA concentrations were determined using a modified Schmidt-Thannhauser method of Fleck and Begg (1965). Tissue samples were precipitated with 0.6M perchloric acid (PCA) and then digested for 1hr at 37°C with 10mls 0.3M sodium hydroxide to hydrolyze the RNA. After cooling, 1.0ml of ice-cold 4.4M PCA was added and the precipitate allowed to flocculate. The mixture was centrifuged and the absorption of the supernatant containing the acid soluble RNA was measured at 260 and 232nm. RNA concentration was calculated using the equation:

$$\underset{(\mu g/ml)}{RNA} = (3.40\ A_{260} - 1.44\ A_{232})\ 10.53$$

The factor 10.53 was used to obtain the true amount of RNA present since uv absorption measures RNA phosphorus rather than RNA itself.

RNA activity was also included in the results sections and was calculated by dividing the rate of protein synthesis by RNA concentration. This measurement gives an indication of how active the RNA is at synthesizing protein.

2.6 PROTEIN SYNTHESIS RATE

The fractional rate of protein synthesis (FSR) was determined by the massive dose method described by Garlick et al (1980). Animals were intraperitoneally injected with 1.0ml/100g bodyweight of L-[4-^{3}H] phenylalanine (80μCi/ml; 150mM in water) and killed by cervical dislocation approximately 10 minutes after injection. The tissues were excised, weighed and frozen immediately in liquid

nitrogen. They were stored at -20°C until analysis.

2.6.1 Tissue Preparation and Extraction

Tissues were prepared by pulverizing them to a fine powder in liquid nitrogen and then homogenized in ice-cold 10% trichloroacetic acid (TCA) using a Polytron homogenizer (Kinematica, Hull). After cooling for 45 minutes the homogenate was centrifuged and the supernatant removed. The protein pellets were washed with ice-cold 10% TCA and the acid supernatant and washings were pooled.

The pooled supernatants were rotary evaporated or lyophilized to dryness then washed twice in deionised water and dried. The supernatant fraction was made up to 3.0mls with 0.5M sodium citrate buffer (pH 6.3). This fraction was used for determination of the specific radioactivity of the free phenylalanine in the tissue samples.

The protein pellets were hydrolysed at 110°C in 6M hydrochloric acid for 22 hours. The hydrolysate was washed and dried in a similar way to the supernatant fraction, dissolved and made up to 3.0mls with sodium citrate buffer (pH 6.3). This fraction was used for determination of the specific radioactivity of the bound phenylalanine in the tissue samples.

2.6.2 Determination of Specific Activities of Phenylalanine

Determination of the specific radioactivities of the free (S_f) and bound (S_b) fractions involved enzymic conversion of phenylalanine to

β-phenylethylamine (β- PEA) followed by extraction of the β-PEA and fluorimetric assay.

Samples of the free or bound fraction (1.0ml) were incubated overnight with 0.5 ml of L-tyrosine decarboxylase apoenzyme (T-4629; Sigma) containing 2 units/ml enzyme (free) or 4 units/ml enzyme (bound). Each enzyme suspension also contained 2mg of pyridoxal 5-phosphate/ml.

β-PEA was extracted from the incubation mixture by shaking in a whirlimixer for 1 minute with 1ml 3M sodium hydroxide and 10mls n-heptane. The organic phase was retained and acidified with 4.0mls 0.01M sulphuric acid. After mixing, the acidic aqueous phase containing the β-PEA was retained.

Determination of the amount of β-PEA was by the fluorimetric method of Suzuki and Yagi (1976) as modified by Garlick et al (1980). One ml of free sample and 0.1ml of bound sample were taken and made up to 1.0ml with 0.01M sulphuric acid as necessary. To this 4.0ml of ninhydrin cocktail (1.0ml 50mM ninhydrin, 0.5ml 2mM L-leucyl-L-alanine, 2.5mls 1M potassium phosphate buffer pH 8.0) were added and mixed. The reaction mixture was kept in the dark. After 60 minutes incubation at 60°C and cooling on ice for 15 minutes the fluorescence was measured at 385nm excitation and 495nm emission wavelengths. The same procedure was performed on duplicate sets of β-PEA at concentrations of 0, 0.3, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 and 20.0 nM to obtain a standard curve.

The radioactivity was measured by liquid scintillation counting and then the specific activities (dpm/nmol β-PEA) calculated for the free and bound fractions.

Fractional synthesis rate was calculated using the equation:

$$FSR\ (\%/d) = \frac{S_b \times 100}{S_f \times t}$$

where t = time in days from injection of the label to freezing the tissue,

S_b = specific activity of the protein bound fraction and

S_f = specific activity of the free fraction.

Prior to experimentation, the specific radioactivity of free phenylalanine was determined in gastrocnemius muscle, liver and small intestine at different times after injection of labelled phenylalanine. Maximum values were reached quickly after injection and followed by a slow, linear fall in specific radioactivity until at least 20 minutes after injection (Fig. 2.6.2.1). Calculation of FSR from a single time was therefore justified.

2.7 PROTEIN DEGRADATION RATE

The rate of protein degradation (FDR) was determined by difference between the rates of protein synthesis and protein growth (FGR) thus:

$$FDR\ (\%/d) = FSR - FGR$$

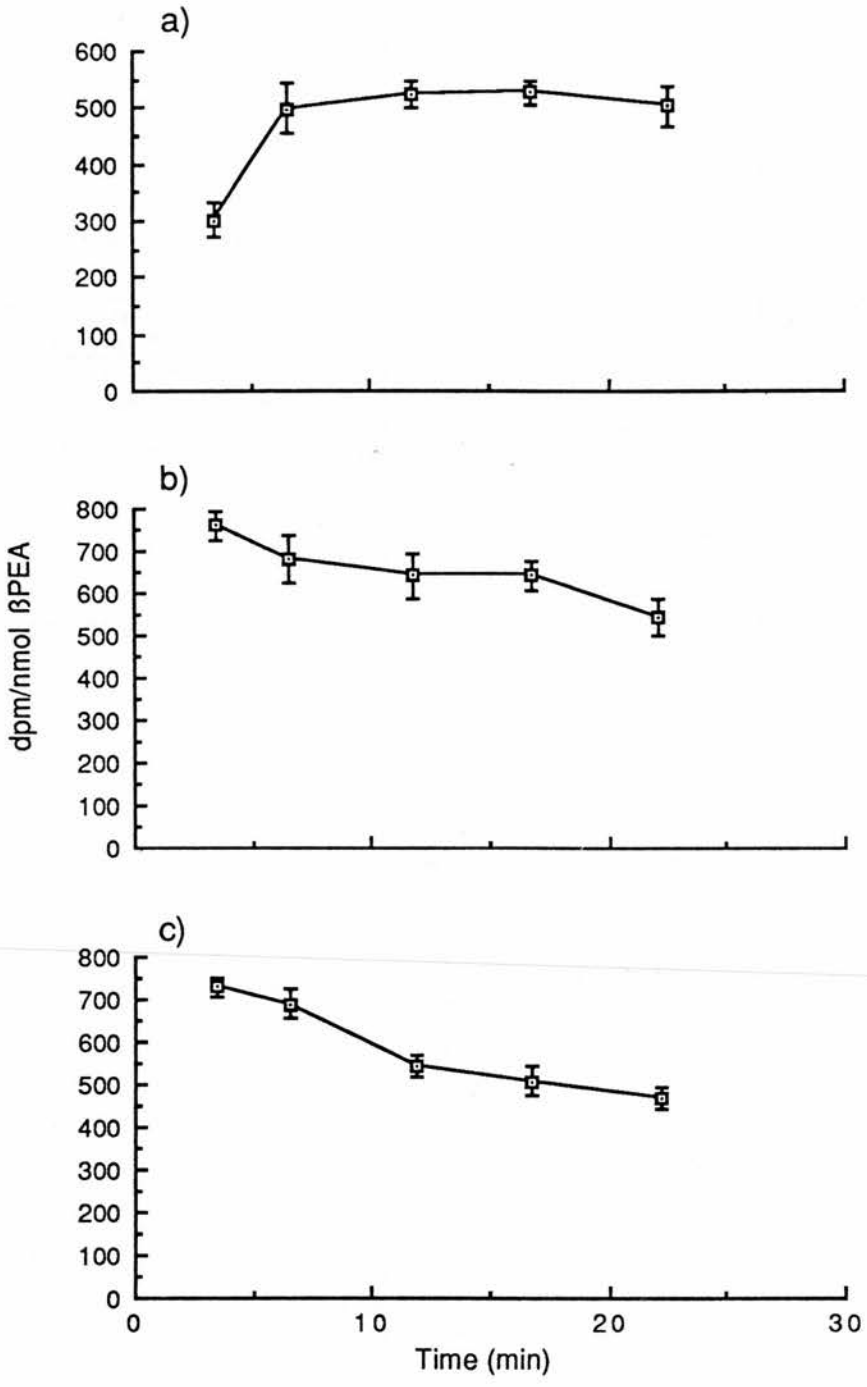


Figure 2.6.2.1 Change in specific activity in tissue of mice selected for appetite at different times after injection of 150 µmol L-[4-^{3}H] phenylalanine. Each point represents the mean ± SEM from eight animals. a) gastrocnemius muscle, b) liver and c) small intestine

The rate of protein synthesis was determined as outlined above and the rate of protein growth by difference between tissue protein contents at two different ages as shown below:

$$\text{FGR} = \frac{P_a - P_b}{n} \times 100$$

(%/d)

where P_a = tissue protein content (mg) of animals older than animals in which FSR measurements were taken,

P_b = tissue protein content (mg) of animals younger than animals in which FSR measurements were taken and

n = difference in age (days) between P_a and P_b groups of animals.

Hence, the instantaneous growth rate was determined and thus the degradation rate.

2.8 LIQUID SCINTILLATION COUNTING

Liquid scintillation counting for [^{3}H] was carried out in an LKB Rackbeta Counter. Disintegrations per minute (d.p.m.) were calculated internally using external standard ratios. Optiphase X was used as scintillation cocktail throughout giving typical counting efficiencies in the range 15-30%.

2.9 THYROID HORMONE DETERMINATIONS

Both total thyroxine (tT_4) and total tri-iodothyronine (tT_3) levels in plasma were measured using Sclavo tT_4 and tT_3 liso-phase radioimmunoassay kits (supplied by Metachem Diagnostics,

Northampton). The kits were a double antibody type RIA with the first antibody (anti-T_4/T_3 serum) being raised in sheep and diluted with 0.1M TRIS buffer (pH 8.6). The second antibody (antisheep-γ-globulins) was bound to Sepharose CL-4B gel in columns (liso-phase columns). Columns were counted on an LKB Wallac 1272 Clinigamma automatic gamma counter. The standard curve and unknown values were calculated and plotted using a previously established programme linked to the counter. Standard curves ranged from 0 to 8.0 ng/ml for tT_3 and 0 to 240 ng/ml for tT_4. Typical unknown plasma values ranged from 0.6 to 2.0 ng/ml for tT_3 and 20 to 120 ng/ml for tT_4.

CHAPTER THREE

RATES OF PROTEIN SYNTHESIS AND DEGRADATION IN MICE SELECTED FOR HIGH AND LOW APPETITE

3.1 INTRODUCTION

Protein metabolism makes a significant contribution towards the body's energy requirements (section 1.2.3). In order to establish if any of the phenotypic differences between mice selected for high or low appetite (section 2.2) were due to metabolic variation in protein turnover the rate of protein metabolism was examined in various tissues of these mice.

The tissues examined were small intestine, liver and gastrocnemius muscle. The gastrocnemius muscle was taken as an example of skeletal muscle. This particular muscle was chosen mainly because it is relatively easy and quick to dissect out of the mouse. These three organs were chosen for several reasons. Firstly, they comprise a major proportion of whole body protein. In a 25kg pig, skeletal muscle makes up 67%, liver 3.2% and intestine 4.9% of whole body protein (Edmunds, Buttery and Fisher, 1978). However, due to different relative rates of protein synthesis their contribution to total protein synthesis is skeletal muscle (35.5%), liver (9.5%) and intestine (36.6%) (Edmunds et al, 1978). In the rat these proportions differ slightly but the liver still makes up approximately 10% of total protein synthesis whereas skeletal muscle accounts for about 20% (Garlick, Burk and Swick, 1976; Garlick, 1980). Thus, these organs are the largest single contributors to whole body protein synthesis (excepting possibly skin and hair) and hence, it would be sensible to examine turnover rates in these tissues. The small intestine was also of particular interest since it is possible that differences in food intake will affect intestinal protein turnover due to the passage of different amounts

of food as well as differences in intestinal wall properties influencing nutrient absorption. The liver may also be closely affected by intakes of different quantities of nutrients and again a change in turnover rates between lines is conceivable. Skeletal muscle is of greatest interest since in meat animals it is the final product and obviously metabolic variations in this organ have critical implications for both meat quantity and quality.

There has been little study of genetic diversity in protein turnover. Evidence so far suggests that strains of mice (Priestley and Robertson, 1973), chicken (Saunderson and Bryan, 1985) and rat (Bates and Millward, 1978) with faster fractional growth rates have a lower rate of protein turnover than their slower growing counterparts (section 1.1.6.2).

In the animals in this study selection was based on differences in food intake and since nutrition influences protein turnover (section 1.2.4) then differences in turnover may occur in the selected animals. Similarly, one would expect this type of selection index to influence the hormonal balance. Various hormonal manipulations can have very drastic effects on the rate of protein turnover (section 1.2.5). Some hormonal preparations were once used as commercial growth promoters and they acted by shifting the balance between protein synthesis and protein degradation.

Thus, in this study protein turnover was investigated in an alternative model for increased growth with increased food intake.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

High and low line mice selected for appetite together with control mice (section 2.2) were either age matched (replicates 1, 2 and 6) at six weeks of age or maturity matched (replicate 3). Mice from generation 21 of selection were used for replicate 2, from generation 23 for replicates 1 and 3 and from generation 27 for replicate 6. Degree of maturity was defined as a given percentage of mature body weight and animals were taken at 73%±6% maturity. Equivalent maturity in the different lines was reached at the same age and so age and maturity matching were regarded as the same. Each group of animals was split into three subgroups. Each subgroup of each replicate contained between 9 and 15 animals depending upon availability. One group was used to determine the fractional rate of protein synthesis with the other two subgroups killed 5 days before and 5 days after this group to measure rate of growth. The rate of protein degradation was calculated from the fractional synthesis and fractional growth rates (FGR).

3.2.2 Methods

The rate of protein synthesis was measured using a flooding dose of a labelled amino acid (Garlick, et al, 1980) and the rate of protein degradation by difference between synthesis and growth rates (Millward et al, 1975). Details of these measurements and of the methods for estimation of RNA, DNA and protein concentrations are given in Chapter 2, sections 2.3-2.8.

3.2.3 Statistical Analysis

Values are given as means of combined replicates with the pooled standard error of the difference. Individual replicate means and standard errors of the means are shown in Appendix 3.

Before statistical analysis, standard deviations versus means were plotted for all subclasses to establish whether the data were distributed normally. On the basis of this information data were transformed to natural logarithms prior to analysis in order that they followed a normal distribution. The statistical tests of significance were assessed by residual maximum likelihood analysis using REML software package (Scottish Agricultural Statistics Service, Edinburgh) which takes into account all sources of variation ie from animals, lines, replicates and replicate by line interactions. Statistical analysis was performed by the Mann-Whitney non-parametric procedure for individual replicates. Behren's test of significance was used for determining mean differences for protein degradation and growth rates since errors in these measurements were due to different causes and estimates of variance cannot properly be pooled.

The degrees of freedom (df) presented in tables 3.3.1, 3.3.2 and 3.3.3 are the lowest possible estimates with the true value lying somewhere between 2 or 3 and 105: the lowest values represent df from lines and the highest value is the df due to total animal number. Therefore, t values rather than probabilities have been shown since probabilities are dependent on the df. Values of t greater than 2.92 are significant ($p<0.05$) with only 2 df and those

greater than 2.35 are significant with 3 df. Thus, when a t value falls between 2 and 3 and the probability is verging towards significance ($p<0.1$) with the minimum df, it has been assumed to be a reasonably good indication of a significant difference. In this situation as few as 4 or 5 df is necessary for a significant difference. When a significant difference was indicated for all the replicates combined, data from individual replicates were examined to either support or refute this hypothesis. This type of analysis is not ideal but interpretation has erred on the pessimistic side since very low df have been used.

Tables in which results from individual replicates have been presented show probabilities rather than t values because the df are unequivocal for individual replicates.

3.3 RESULTS

Body and Organ Weights.

High line body, liver and small intestine weights were invariably heavier than C lines which in turn were heavier than L line groups at six weeks of age (Table 3.3.1, individual replicates in Appendix 3.3.1A). Mean muscle weights were not shown to differ when replicates were combined yet three out of four individual replicates showed a highly significant difference between H and L groups (H>L). Mean muscle weights did not differ in replicate 1 (Appendix 3.3.1A). Results are thus in general agreement with those of Sharp *et al* (1984) who found that growth was proportional.

Table 3.3.1 Body and organ weights of mice divergently selected for 4-6 week food intake (combined replicates).

	High			Control			Low			pooled $l_n x$		t		
	x	$l_n x$	n	x	$l_n x$	n	x	$l_n x$	n	sed	df	H-L	H-C	C-L
Body weight (g)	29.6	3.39	40	25.5	3.24	23	23.1	3.14	46	.0271	3	9.16	5.58	3.58
Muscle weight (g)	.133	-2.02	40	.109	-2.22	23	.111	-2.19	46	.1171	3	1.51	1.74	-0.23
Liver weight (g)	1.843	.6116	32	1.663	.5086	23	1.397	.3340	32	.0359	2	7.73	2.87	4.86
SI weight (g)	1.405	.3403	34	1.307	.2675	23	0.995	-.047	33	.0428	2	8.06	1.70	6.36

SI = small intestine and n = number of animals per group.

Protein and Nucleic Acid Concentrations.

There was no change in any tissue protein concentration (Table 3.3.2) therefore the greater protein contents in H than L appetite mice (Table 3.3.2) reflect organ weight differences. However, in the C line this measurement was not always midway between H and L values. In muscle, C line total protein content was very close to that of the L group whereas in the liver, H and C protein contents were very similar. No consistent variation was evident in either the RNA or DNA concentrations per g protein of any tissue examined (Table 3.3.2). However, there was a non-significant trend in replicates 2,3 and 6 for RNA concentration to be higher in L line animals (Table 3.3.2A).

Protein Synthesis

The FSR in the gastrocnemius muscle of the H line was significantly lower than that in L appetite mice (Table 3.3.3). The C line FSR was very similar to that in the H line and so it appears that selection increased the rate of protein synthesis in skeletal muscle of L appetite mice. Upon examination of individual replicates (Table 3.3.3A) as with muscle weight, replicate 1 differed from the other three replicates. In this replicate the skeletal muscle FSR tended to be greater in the H line than C and L lines. In the liver the trend was for a decrease in FSR in both H and L groups to a value below that in C mice. No difference due to selection was apparent in the FSR of the small intestine. The RNA activities in all three tissues tended to reflect differences in the rates of protein synthesis and although in the case of the liver

Table 3.3.2 Gastrocnemius muscle, liver and small intestine protein, RNA and DNA concentrations and total organ protein contents of mice divergently selected for appetite (combined replicates).

		High			Control			Low			pooled $l_n x$		Test of significance		
		x	$l_n x$	n	x	$l_n x$	n	x	$l_n x$	n	sed	df	H–L	H–C	C–L
Prot. conc. (mg/g)	M	180.9	5.198	40	176.6	5.174	23	183.5	5.212	46	.0656	3	-0.21	0.37	-0.58
	LI	147.2	4.992	32	160.0	5.075	23	144.5	4.973	32	.0546	2	0.35	-1.52	1.87
	SI	123.2	4.814	34	114.7	4.742	23	108.6	4.688	33	0.141	2	1.04	0.51	0.53
RNA conc. (μg/mg prot)	M	7.29	1.99	40	7.91	2.07	23	7.16	1.97	46	0.110	3	0.16	-0.75	0.90
	LI	8.94	2.19	32	8.63	2.16	23	8.42	2.13	32	0.026	2	2.29	1.33	0.95
	SI	9.73	2.28	34	10.85	2.38	23	9.48	2.25	33	0.052	2	0.50	-2.08	2.58
DNA conc. (μg/mg prot)	M	4.09	1.41	40	5.87	1.77	23	4.51	1.51	46	0.126	2	-0.78	-2.86	2.09
	LI	1.23	.210	32	1.41	.341	23	1.22	.201	32	0.098	2	0.09	-1.35	1.44
	SI	5.74	1.75	34	5.77	1.75	23	5.60	1.72	33	0.050	2	0.50	-0.08	0.58
Total prot per organ (mg)	M	24.2	3.19	40	19.0	2.95	23	18.9	2.94	46	0.117	3	2.10	2.04	0.07
	LI	271.5	5.604	32	265.9	5.583	23	200.9	5.306	32	0.067	2	4.44	0.31	4.13
	SI	180.4	5.195	34	150.4	5.013	23	105.0	4.654	33	0.150	2	3.60	1.21	2.39

M = gastrocnemius muscle, LI = liver, SI = small intestine and n = number of mice/group.

Table 3.3.3 Rates of protein synthesis and RNA activities of various tissues from mice selected for 4-6 week food intake (combined replicates).

		High			Control			Low			pooled $l_n x$		Test of significance		
		x	$l_n x$	n	x	$l_n x$	n	x	$l_n x$	n	sed	df	H-L	H-C	C-L
FSR (%/d)	M	15.2	2.72	40	15.0	2.71	23	18.2	2.90	46	0.085	3	-2.17	0.11	-2.28
	LI	97.7	4.58	32	116.9	4.76	23	91.5	4.52	32	0.086	2	0.77	-2.09	2.86
	SI	79.5	4.38	34	84.3	4.43	23	85.4	4.45	33	0.054	2	-1.30	-1.07	-0.24
RNA act (FSR/ RNA)	M	2.08	.732	40	1.81	.594	23	2.43	.889	46	0.133	3	-1.18	1.04	-2.22
	LI	1.64	.496	32	2.12	.753	23	1.66	.509	32	0.139	2	-0.10	-1.86	1.76
	SI	1.03	.025	34	0.90	-.110	23	0.96	-.045	33	0.195	2	0.36	0.69	-0.33

M = gastrocnemius muscle, LI = liver, SI = small intestine and n = number of mice/group.

this was not significant, the general trend was the same as the FSR measurements.

Protein Degradation.

The rates of protein degradation in gastrocnemius muscle (Table 3.3.4) reflected differences in the rates of protein synthesis and were usually higher in the L line than the other groups but again upon examination of individual replicates (Table 3.3.4A) replicate 1 tended to show an opposite difference. Although liver and small intestine rates of protein degradation followed the trends in synthesis rates, no differences were significant. In all tissues FGRs were similar between groups which meant that the amount of protein accreted per day should be closely associated with tissue weights. Therefore, in H line muscle, the amount of protein accreted per day tended to be greater than that in L lines and the tendency was the same in liver although the difference was not significant. No trend in the amount of protein accreted was evident in small intestine despite a line difference in small intestine weight. It is probable that the small intestine had reached its mature weight by this stage.

3.4 DISCUSSION

The observed differences in protein synthesis between H, C and L mice allow some conclusions to be drawn concerning the efficiency of protein synthesis and deposition. Unfortunately, because the rate of protein degradation could not be measured directly and was calculated from two average measurements, one of which was itself

Table 3.3.4 Fractional rates of protein degradation and growth and amount of protein accreted per organ in various tissues of appetite selected mice.

			High		Control		Low		Test of significance		
		Rep.	x	sem	x	sem	x	sem	H-L	H-C	C-L
FDR (%/d)	M	1	14.6	1.6	8.2	1.8	13.6	1.5	NS	*	*
		2	11.8	3.1	-	-	22.0	3.2	*	-	-
		3	6.0	4.0	7.5	6.5	9.8	5.8	NS	NS	NS
		6	16.8	2.7	-	-	27.2	5.2	NS	-	-
	LI	1	81.5	7.7	106.4	6.3	96.2	9.7	NS	*	NS
		3	88.8	4.1	95.4	5.2	75.7	6.6	NS	NS	*
		6	143.7	13.5	-	-	122.6	10.8	NS	-	-
	SI	1	74.8	4.0	80.6	5.5	83.6	5.3	NS	NS	NS
		3	81.7	4.4	77.1	4.0	70.2	5.6	NS	NS	NS
		6	87.3	6.0	-	-	106.4	6.9	NS	-	-
FGR (%/d)	M	1	2.4	1.0	5.7	1.3	0.8	1.2	NS	*	**
		2	5.2	1.0	-	-	3.0	1.1	NS	-	-
		3	3.9	3.9	3.4	6.4	3.1	5.7	NS	NS	NS
		6	3.3	1.2	-	-	3.9	1.6	NS	-	-
	LI	1	2.2	4.8	0.7	1.2	1.2	7.6	NS	NS	NS
		3	3.3	1.1	3.9	1.0	3.1	1.3	NS	NS	NS
		6	-0.3	7.6	-	-	1.8	1.1	NS	-	-
	SI	1	-0.9	1.1	1.3	1.3	-1.0	2.4	NS	NS	NS
		3	0.7	0.8	1.4	1.0	2.4	1.2	NS	NS	NS
		6	1.0	0.7	-	-	0.5	0.9	NS	-	-
Protein accreted per organ (mg)	M	1	0.5	0.2	1.2	0.2	0.2	0.3	NS	*	*
		2	1.2	0.3	-	-	0.6	0.2	*	-	-
		3	1.2	0.2	0.7	0.1	0.7	0.2	NS(*)	*	NS
		6	0.8	0.2	-	-	0.7	0.3	NS	-	-
	LI	1	5.6	1.5	1.7	2.2	2.3	1.0	NS	NS	NS
		3	11.3	3.8	17.2	3.9	9.4	3.3	NS	NS	NS
		6	-0.8	1.8	-	-	3.0	1.8	NS	-	-
	SI	1	-1.6	1.5	2.0	1.1	-0.9	0.7	NS	NS	*
		3	1.5	2.0	2.8	1.3	3.5	1.2	NS	NS	NS
		6	-1.6	1.1	-	-	0.6	1.1	NS	-	-

M = gastrocnemius muscle, LI = liver and SI = small intestine.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$ and ** = $p<0.01$.

calculated from two means, then the errors accumulated and there were no significant differences between lines. If a direct method of measuring protein degradation had been available one would have expected rates to reflect the differences observed in protein synthesis. Protein synthesis was greater in the muscle of L than C and H line mice although the muscle weight and protein content per muscle were lower than in the H group. It follows firstly, that the maintenance rates of protein synthesis and degradation were greater ie basal protein turnover was less efficient, in L line mice than either C or H mice. In addition, the amount of protein accreted by the H line group was greater than that laid down by either the C or L mice and therefore, the efficiency of protein synthesis and degradation associated with protein deposition was greater in the H line than either C or L mice. Hence, skeletal muscle protein deposition in the L group seems to be less efficient than in C animals because they have an elevated maintenance turnover rate but the H line was more efficient due to a greater accretion rate at a similar level of turnover to the C mice. This contrasts with the findings of Bishop and Hill (1985) who reported that high intake appetite line mice had higher maintenance requirements for the whole body than low intake mice. This discrepancy between whole body and skeletal muscle measurements implies that other tissues or some other major process that uses energy has much elevated maintenance requirements. Protein turnover in neither the liver nor the small intestine are likely candidates since synthesis rates were similar in the liver of both H and L groups as well as between H, C and L groups for small intestine. This indicates that no differences in maintenance requirements were due to variation in protein metabolism in these tissues with the exception of C group liver in which the

turnover rate was elevated above H and L counterparts. Maintenance energy requirements would therefore, be slightly elevated in this tissue in C mice but this would be unlikely to have a major effect upon the whole body maintenance energy requirements.

Differences in rates of protein synthesis in skeletal muscle appear to be correlated with RNA activity with an indication that RNA capacity may also be involved. This is interesting because the major cause of changes in protein synthesis due to age or nutrition is usually an alteration in RNA capacity with only very acute and minor changes being attributed to adaptation in RNA activity (Millward et al, 1973; Millward, Nnanyelugo and Garlick, 1974b; Goldspink and Kelly, 1984). Perhaps changes in protein synthesis due to selection are much less severe than those due to age or nutrition and therefore a milder adaptive change in RNA parameters occurs.

The lack of variation in DNA concentration indicates that the amount of cytoplasm associated with each "nucleus" in muscle syncitia is constant. Therefore, since larger amounts of protein were deposited in H line muscle, then the number of "nuclei" per muscle must also have increased. The same arguement also applies to liver and small intestine cells. Since DNA concentration was not different between lines then the larger H line organs must have a greater number of cells.

It is interesting to compare the effects of nutrition with the effect of selection for appetite upon protein turnover. In the rat it has been established that protein deprivation and starvation

cause a decline in both the rates of protein sythesis and degradation with an accompanying loss of skeletal muscle protein (section 1.2.4; Millward et al, 1973; Garlick et al, 1975). This study showed mice with lower appetites to have a greater rate of skeletal muscle protein turnover and a lower rate of protein deposition. Obviously these two situations are not directly equivalent because one situation is the involuntary reduction in food intake and the other is an innate lowering of the appetite with only a reduction in the rate of muscle growth rather than absolute loss of muscle protein. Nevertheless, it appears that selection for reduced food intake has opposite effects on the rate of protein turnover to imposed reductions in food intake.

This study demonstrated that the faster growing H line had the lower muscle protein synthesis and degradation rates than the slower growing L line. Bates and Millward (1978, 1981) compared the fractional protein growth and breakdown rates in slow growing and fast growing strains of rat. In agreement with the experiment reported here, the fast growing CFY strain had lower muscle protein synthesis and degradation rates than the slow growing hooded rat strain.

Kielanowski (1968) hypothesized that selection of animals for high weight gain on a set level of feeding would favour those that deposited more metabolizable energy as protein rather than fat. To deposit 1g fat and protein each requires approximately 55kJ/g (Webster, 1977; section 1.2.3). However, protein accretion is also associated with water deposition as lean tissue and so per unit body weight gain, lean deposition rather than fat is more efficient.

This was confirmed by McPhee and Trappett (1987) where mice selected for 3-6 week body weight gain on a set feeding level partitioned the retained energy in favour of more protein but ad libitum selected mice favoured more fat deposition. In comparison, the H lines of this study were slightly leaner (Bishop and Hill, 1985) than their L line counterparts although McPhee and Trappett's (1987) comparable ad libitum selection line were fatter. As well as differences in the division of retained energy between fat and protein (Hetzel and Nicholas, 1978; McPhee et al, 1980; McPhee and Trappett, 1987) it has also been shown that both set feeding and ad libitum selected lines have reduced maintenance requirements compared to control animals. These results concur with skeletal muscle protein turnover in the high appetite line but not with the measure of whole body maintenance requirements. It would be interesting to observe how H mice responded if they were restricted to L type quantities of food.

Replicate 1 often appeared to show results which were the converse of other replicates. The aim of selection (changed appetite) therefore appears to have been achieved by different metabolic strategies in the different replicates. This is good illustration of the reasons for replicating selection experiments.

The consistency or repeatability of selection experiments depends partly upon sampling variation which is affected by factors such as population size and initial gene frequencies. Hence, in this case the circumstances were such that the genes involved in protein metabolism were not very strongly linked to those for appetite and sampling variation made this evident.

If replicate 1 was excluded from the data analysis, the rate of muscle protein synthesis was significantly lower in the H than L line ($p<0.05$, H = 14.6 and L = 21.2 %/d) even using the lowest df since the t value was -3.763. Therefore, it seems that replicate 1 does considerably affect the statistical analysis.

In summary, the major finding of this study has been that the rate of skeletal muscle protein synthesis was significantly lower in H than L appetite mice and that the efficiency of protein deposition in this tissue was greater in the H than L line.

CHAPTER FOUR

THYROID HORMONE CONCENTRATIONS IN MICE SELECTED FOR HIGH AND LOW APPETITE

4.1 INTRODUCTION

The previous experiment (Chapter 3) showed that H appetite mice had a significantly lower rate of skeletal muscle protein turnover than L animals. Many hormones and factors affect protein turnover and so to try and establish a link between this difference and a particular hormone is very difficult. However, it was decided to investigate thyroid hormone levels in the different lines in the first instance merely to discover if their concentrations varied between groups.

Firstly, thyroid hormones affect protein turnover and growth. It is well documented that thyroid hormones increase rates of protein synthesis and degradation in skeletal muscle (section 1.3.3.2; Carter et al, 1976; De Martino and Goldberg, 1978). Therefore, if these hormones were one of the major causes of the different protein turnover rates one may expect lower thyroid hormone concentrations in H than L appetite mice. Differences in levels of TBG and receptor concentrations may also be altered without differences in concentrations of thyroid hormones but examining blood hormone concentrations is the first parameter to study. Thyroid hormones also seem to be associated with higher growth rates (section 1.3.3.2; Carter et al, 1982; Martynenko, 1984; Bates and Holder, 1986) yet H line mice with the lower rate of protein turnover also had faster growth rates!

Secondly, thyroid hormones have been linked with appetite and food intake. For instance, overnutrition tends to increase and undernutrition to decrease the concentration of thyroid hormones in

the blood (Millward et al, 1979; Ortiz-Caro et al, 1984). In these animals which exhibit growth, protein turnover and appetite differences it therefore seemed appropriate to investigate circulating thyroid hormone concentrations although the expected effects of thyroid hormones on protein turnover and appetite are contradictory (ie mice with a higher appetite had a lower rate of skeletal muscle protein turnover).

Thyroid hormone concentrations were determined in replicates 1, 2 and 3 at five weeks of age and replicates 1, 2, 3 and 6 at six weeks of age.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design

At five weeks of age male mice from replicates 1, 2 and 3 (generation 18; samples kindly donated by Miss P.J. Cook, Dept. of Genetics, University of Edinburgh) were taken for blood sampling and at six weeks of age male mice from all four replicates were used (generation 23, replicates 1, 2 and 3 and generation 27, replicate 6). At five weeks of age animal numbers in replicate 1 ranged from 9-12 animals per group, in replicate 2 from 5-6 animals and in replicate 3 from 11-30 animals. At six weeks of age animal numbers in replicate 1 were 20-27 per group, in replicate 2 from 18-27, in replicate 3 from 19-20 and in replicate 6 from 34-35. As many animals were used as were available. Samples from all lines were taken. Animals were weighed on the day of sampling. Blood was taken by cardiac puncture under terminal anaesthesia using syringes

washed in heparinised saline (100iu/ml saline). Samples were immediately placed on ice. Plasma was separated by centrifugation and stored at -20°C until analysed.

4.2.2 Methods

Both tT_4 and tT_3 concentrations were determined in all the plasma samples using Sclavo RIA kits as described in section 2.9.

4.2.3 Statistical Analysis

Means of the combined replicates are given with the standard error of the difference. Means and standard errors of the means are shown for individual replicates. On the basis of information obtained from plotting standard deviations versus means for all subclasses, data from the six week groups was transformed to natural logarithms before analysis so that data followed a normal distribution whereas the raw data was used for samples taken at five weeks of age.

Statistical tests of significance for combined replicates were performed by residual maximum likelihood analysis using REML software package (Scottish Agricultural Statistics Service, Edinburgh). Values of t rather than probabilities have been presented due to the imprecise estimates of the degrees of freedom as explained previously (section 3.2.3). The degrees of freedom shown are minimum estimates. The Mann-Whitney procedure was used to determine whether differences were significant for individual replicates. Probabilities are shown for individual replicates because the df are not equivocal.

4.3 RESULTS

Body Weight.

As expected all H appetite groups had a heavier body weight than both their C and L line counterparts. At five weeks of age the body weight of the C line was very close to that of the L group (Table 4.3.1 and Table 4.3.1A for individual replicates) whereas at six weeks of age C and L line body weights were significantly different (Table 4.3.2 and Table 4.3.2A for individual replicates).

Thyroid Hormone Concentrations.

Total T_4 concentrations in the H line mice were found to be significantly lower than either C or L counterparts at five weeks of age (Table 4.3.1). At six weeks of age this difference persisted between H and C groups but the difference between H and L groups was much reduced (Table 4.3.2). No difference in tT_3 concentrations was apparent between any group at either age tested (Tables 4.3.1 and 4.3.2). Examination of individual replicates at five weeks of age (Table 4.3.1A) showed that the relationship between tT_4 and line was not followed by replicate 1.

4.4 DISCUSSION

There is a strong indication that tT_4 concentrations are lower in H than L groups. Similar results were obtained in a study in which faster growing Sprague Dawley rats were shown to have significantly lower circulating T_3 and T_4 levels than slower growing Wistar rats

Table 4.3.1 Body weights and plasma thyroid hormone concentrations at five weeks of age in mice selected for appetite (combined replicates).

	Mean					Test of significance		
	High (45)	Control (34)	Low (26)	pooled sed	df	H-L	H-C	C-L
Body weight (g)	27.0	23.5	24.1	0.916	2	3.14	3.81	-0.67
Total T_4 (ng/ml)	44.9	59.4	58.0	3.60	2	-3.63	-4.03	0.41
Total T_3 (ng/ml)	0.95	0.86	1.01	0.104	2	-0.59	0.86	-1.45

Numbers in brackets ( ) represents animals per group.

Table 4.3.2 Body weights and plasma thyroid hormone concentrations at six weeks of age in mice selected for appetite (combined replicates).

	High (106)		Control (57)		Low (100)		pooled $l_n x$		Test of significance		
	x	$l_n x$	x	$l_n x$	x	$l_n x$	sed	df	H–L	H–C	C–L
Body weight (g)	28.6	3.35	27.2	3.31	23.3	3.15	.0221	3	9.256	2.123	7.134
Total T_4 (ng/ml)	48.7	3.89	54.4	4.00	50.2	3.92	.03418	3	-0.934	-3.345	2.401
Total T_3 (ng/ml)	1.11	.107	1.08	.081	1.10	.091	.0570	3	0.28	0.46	-0.18

Numbers in brackets ( ) represents animals per group.

(Kuhn et al, 1983). In addition, newborn Ossabaw piglets were shown to have a higher plasma T_4 concentration than heavier newborn Yorkshire piglets (Kasser et al, 1981). The percentage $^{[131]}I$ uptake and turnover per metabolic body weight were also greater in mice selected for low six week body weight than their high line counterparts (Synenki et al, 1972). However, when $^{[131]}I$ uptake was not scaled to metabolic body weight then the high line showed highest $^{[131]}I$ uptake and turnover by the thyroid.

In contrast, mice selectively bred for high $^{[131]}I$ uptake into the thyroid also had a greater body weight and growth rate than low line counterparts (Chai, 1970). This relationship still held with approximately a four fold difference when $^{[131]}I$ uptake was expressed per metabolic body weight. Other studies have also shown a positive correlation between high growth rates and body weights with parameters of thyroid metabolism (Stewart and Washburn, 1983) although others have not found any connection between these parameters (Hurst and Turner, 1948; Silverstein et al, 1960; Edwards, 1962). These earlier studies indicate that thyroid hormones may not be necessary for a high growth rate but they may be involved in some cases. However, in this experiment lower thyroid hormone concentrations appeared to be connected with higher growth rates. It is not clear whether thyroid function is important in determining growth rates in these mice. It is important to realise that total thyroid hormone concentrations rather than free thyroid hormone concentrations were measured in this experiment. Therefore, if the selection process had also changed TBG or TBPA levels as well as thyroid hormone concentrations, then trends indicated by total thyroid hormone concentrations may not necessarily be followed by

free hormone levels. Hence, the results obtained in this study must always be interpreted with this in mind.

Replicate 1 again showed differences converse to the other replicates as in the previous experiment (Chapter 3). This supports evidence to suggest that selection achieved the same end result of increased appetite in this replicate by acting upon metabolic pathways in different ways to the other replicates.

There is a positive correlation between the rate of skeletal muscle protein synthesis and degradation and thyroid hormones (Carter et al, 1980). Therefore, since the rate of muscle protein synthesis was lower in the H than L line mice, (Chap. 3) the thyroid hormone concentrations would also be expected to be less in the H animals. This relationship was observed in this experiment. However, it does not appear that differences in appetite can be attributed to differences in thyroid hormone concentrations because thyroid hormones usually increase appetite. Thus, differences in thyroid hormone concentrations may contribute towards differences in rates of protein synthesis and degradation in skeletal muscle of mice selected for appetite but they do not appear to influence appetite.

CHAPTER FIVE

RESPONSE OF PROTEIN TURNOVER IN HIGH AND LOW APPETITE MICE TO THYROIDECTOMY AND THYROXINE REPLACEMENT

5.1 INTRODUCTION

In Chapters 3 and 4 it was demonstrated that muscle protein turnover and thyroid hormone concentrations differed between H and L appetite mice. It is well known that increasing thyroid hormone concentrations can affect both protein synthesis (Mathews et al, 1973; Carter et al 1976; Brown et al, 1981) and protein degradation (De Martino and Goldberg, 1978; Goldberg et al, 1980) tending to increase their rates. The overall result of raised thyroid hormone levels is usually an increased rate of whole body growth (Carter et al, 1982; Martynenko and Kornyushenko, 1984; Bates and Holder 1986) due to protein synthesis rates being increased to a larger extent than degradation rates. However, it has been reported that in cases of excessive hyperthyroidism growth rates can be depressed (Scow, 1954).

In H line animals thyroid hormone concentrations and protein turnover rates of the skeletal muscle protein were decreased which may indicate the involvement of thyroid hormones in governing the processes of protein turnover in appetite-selected mice. However, an important role for thyroid hormones is not indicated by the observed growth differences since decreased thyroid hormone levels tend to depress growth rates which is clearly not so in this case. This contradiction can be resolved by the reported local modulation of thyroid hormone action whereby their effects can either be attenuated or amplified in different tissues (Gaspard, Wondergem and Klitgaard, 1975). By this means it may be possible that thyroid hormones are involved in differential changes in protein turnover in different tissues.

The possible role of thyroid hormones in regulating protein turnover in appetite-selected mice was studied in an experiment in which mice were thyroidectomized (TX) and then given either saline or a replacement dose of T_4 at a set level. Thyroxine rather than T_3 was used to mimic the body's natural circulating thyroid hormones better because circulating T_4 is peripherally converted to T_3 and so both T_4 and T_3 would be present in the blood. Injection of T_3 however, would have excluded the presence of the prohormone, T_4, from the circulation. The rates of protein synthesis and breakdown were then determined in skeletal muscle and liver. Comparison of rates of protein turnover between lines on the same treatment should give an indication of whether or not thyroid hormones are involved in regulation. In addition, if thyroid hormones are involved in regulation, hormone replacement studies would indicate whether this involvement was by variations in circulating hormone concentrations or in tissue responsiveness to the hormones.

5.2 MATERIALS AND METHODS

5.2.1 Animals: housing and management

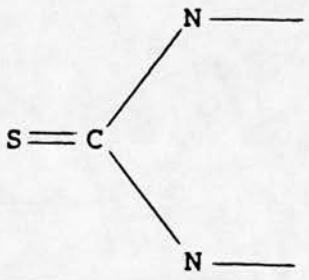
Male mice from the combined selection replicate, replicate 6, were housed at 20°C on a natural lighting regimen and had free access to food and water. They were grouped and caged in twos or threes. Only mice from replicate 6 were used since this replicate represents the other three replicates combined and obtaining sufficient mice from all the replicates would have been very difficult in the time available. For at least three days prior to experimentation mice were allowed to acclimatize to the cages and were also weighed daily

to become accustomed to being handled.

5.2.2 Thyroidectomy and Thyroid Replacement

5.2.2.1 Thyroidectomy

A chemical thyroidectomizing agent, methimazole (1-methyl-2-mercaptoimidazole) was used. Methimazole is a thioureylene antithyroid drug that inhibits iodination of thyroglobulins in the thyroid gland and so prevents T_3 and T_4 formation (Taurog, 1976). The thioureylene grouping is:



Methimazole antithyroid action is reversible and so the drug was administered daily.

To ascertain the necessary methimazole dosage required to reduce T_4 to undetectable levels a preliminary dose response trial was set up. Mice were administered either 1.0, 2.0, 4.0 or 16.0 mg methimazole per day in 0.1ml water by intraperitoneal injection for one, two, three or four days. Total T_4 levels in plasma were determined (section 2.9). Results are presented in Table 5.2.2.1.1. The minimum detectable concentration of T_4 significantly different

Table 5.2.2.1.1
Mouse plasma tT_4 concentrations (ng/ml) upon daily methimazole administration at various doses (1.0, 2.0, 4.0 and 16.0 mg/d) from zero to four days duration.

MMI dosage (mg/d)	Days of administration				
	0 (2)	1 (2)	2 (3)	3 (3)	4 (2)
1.0	44.3	44.8	49.4	40.3	39.8
2.0	56.1	21.6	18.8 / -	23.5 2-	45.4 /
4.0	51.9	21.1	3-	19.9	25.5
16.0	51.8	20.9	3-	-, 2/	2/

Numbers in parentheses represents number of animals per group.

MMI = methimazole.

- = undectectable tT_4 levels with the figure indicating the number of mice with undetectable tT_4 levels.

/ = group numbers diminished by death with the figure indicating the number of animals dead.

from zero was 15.0ng/ml ($p<0.05$). On the basis of this trial a dose of 4.0mg methimazole per day was selected for the main trial since this was the dose at which minimum thyroid hormone concentrations were consistently reached without adverse effects.

For the main trial methimazole was dissolved in 0.9% saline to a concentration of 40mg/ml. Fresh solutions were prepared every three or four days. Mice received 4mg daily by intraperitoneal injection.

5.2.2.2 Thyroid Replacement

L-thyroxine (sodium salt pentahydrate T-2501, Sigma) was dissolved directly in 0.9% saline to a concentration of 80μg/ml. Fresh preparations were made every four to six days. Mice were injected daily in the subcutaneous neck skinfold with 8μg/d. This level of T_4 administration was chosen after examination of the literature where T_4 replacement doses have been studied (Holder and Wallis, 1977; Van Buul Offers et al, 1984; Bates and Holder, 1986). These studies looked at the effect of T_4 administration on the growth of Snell dwarf mice compared with normal littermates. Dose rates varied between 0.1 and 8.0μg/d and were administered subcutaneously with a good growth response and differences in the rate of skeletal muscle protein synthesis being obtained when 8μg/d T_4 was administered (Holder and Wallis, 1977; Bates and Holder, 1986). Thus, 8μg/d was used in this experiment.

5.2.3 Experimental Design

Male H and L appetite mice of generation 28 were each divided into two groups for treatment with either T_4 or saline. Each of these groups was further divided to form three subgroups. One subgroup was used for determination of the fractional rate of protein synthesis at 42 days of age and the remaining two subgroups were used for determination of the FGR (killed at 37 and 47 days) and hence the FDR. There were between 7 and 10 animals per group. Ideally a group of animals for each line with no manipulation except sham thyroidectomy would also have been included in this study but difficulty in obtaining sufficient numbers of animals prevented this. Using different T_4 doses was excluded for the same reason.

The mean initial weight of the H appetite groups was 16.6g (range 10.9-21.8g) and the L appetite groups was 13.1g (range 9.5-19.5g). Between 8.00 and 10.00 am all animals received daily methimazole injections from 30 days of age. At 32 days of age, designated as day 0 of the trial, either daily T_4 (8μg/d in 0.1ml saline) or saline (0.1ml/d) injections were also administered. Animals were weighed and the food consumption was recorded at the time of injection. On days 5 and 15 of the trial (at 37 and 47 days of age respectively) groups of mice from each treatment were killed and the gastrocnemius muscle (including plantaris) and liver were excised, weighed and frozen. At 42 days of age, day 10 of the trial, groups from each treatment were taken for measuring the rate of protein synthesis (section 2.6). Mice were intraperitoneally injected with L-[4-^{3}H] phenylalanine (1.0ml/100g body weight, 80μCi/ml, 150mM in water, see section 2.6) and left for ten minutes. After being

killed by cervical dislocation, the gastrocnemius muscle (including plantaris) and liver were quickly dissected out, weighed and frozen in liquid nitrogen. Blood was not collected for measurement of thyroid hormone concentrations because this would have delayed tissue dissection and freezing for too long. All tissues were stored at -20°C until analysis. The time from injection of each mouse with L-[4-^{3}H] phenylalanine to when the individual tissues were frozen in liquid nitrogen was accurately recorded. Samples taken on days 5 and 15 of the trial were analysed for protein concentration (section 2.3) and those collected on day 10 for protein, RNA (section 2.5) and DNA (section 2.4) concentrations and for the rate of protein synthesis (section 2.6).

5.2.4 Statistical Analysis

Means are presented with standard errors. Statistical analyses for different lines and treatment groups were performed by Mann-Whitney rank test. Combined groups (H v L, Saline v T_4) statistical analyses were carried out by two-way analysis of variance by rank.

5.3 RESULTS

Body and Tissue Weights.

The body weight and tissue weights did not differ between saline and T_4-treated mice (Table 5.3.1) except the liver of the saline-treated L appetite group was heavier than its T4-treated counterpart. There was a similar trend in the H appetite line. All

Table 5.3.1

Body and organ weights of appetite selected mice upon thyroidectomy or replacement T_4 administration.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.
Parentheses indicate the number of animals per group.

Table 5.3.1
a)

	HIGH				LOW				Test of significance			
	Saline (7)		T_4 (7)		Saline (7)		T_4 (9)		HS v HT	LS v LT	HS v LS	HT v LT
	x	sem	x	sem	x	sem	x	sem				
Body weight(g)	21.0	1.2	21.5	1.2	15.5	0.6	14.7	1.2	NS	NS	*	**
Muscle weight(g)	0.089	0.008	0.095	0.012	0.063	0.004	0.059	0.005	NS	NS	*	*
Liver weight(g)	1.505	0.088	1.414	0.087	1.075	0.055	0.841	0.061	NS	*	**	***

b)

	Saline (14)		T_4 (16)		High (14)		Low (16)		Test of significance	
	x	sem	x	sem	x	sem	x	sem	S v T_4	H v L
Body weight(g)	18.2	1.0	17.7	1.2	21.2	0.8	15.0	0.7	NS	***
Muscle weight(g)	0.076	0.006	0.075	0.007	0.092	0.007	0.061	0.003	NS	**
Liver weight(g)	1.290	0.078	1.093	0.089	1.460	0.061	0.940	0.235	NS(*)	***

H appetite groups had heavier body and tissue weights than their L line counterparts which agrees with previous results (section 3.3; Bishop and Hill, 1985).

Protein, RNA and DNA Concentrations.

There was neither a treatment nor a line difference in protein concentrations of the two tissues (Table 5.3.2). Therefore, the total amount of protein in each tissue reflected the differences in tissue weights. There was no difference in the tissue DNA concentration between treatments but in the liver there was a line difference (Table 5.3.2). RNA concentration showed no significant variation between lines but it was significantly higher ($p<0.01$) in the liver of T_4-treated H animals than in saline-treated counterparts (Table 5.3.2). In L animals a similar but not significant trend was observed although when lines were combined the difference was significant.

Rates of Protein Synthesis and Degradation and RNA Activity.

No change was apparent in either muscle protein synthesis rate or RNA activity either between lines or treatments although the protein synthesis rates tended to be lower in the T_4 versus saline-treated mice (Table 5.3.3). Liver protein synthesis rates and RNA activities were not shown to differ but a pattern emerged with the rates of protein synthesis in both T_4-treated groups tending to be greater than their saline-treated counterparts (Table 5.3.3). All liver RNA activities were very similar (Table 5.3.3). The rates of protein degradation reflected the rates of protein synthesis in all

Table 5.3.2

Protein, RNA and DNA concentrations and protein contents of various tissues in thyroidectomized and T_4 replacement treated appetite selected mice.
M = gastrocnemius muscle and LI = liver.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.
Parentheses indicate the number of animals per group.

Table 5.3.2
a)

		High				Low				Test of significance			
		Saline (7)		T_4 (7)		Saline (7)		T_4 (9)		HS v HT	LS v LT	HS v LS	HT v LT
		x	sem	x	sem	x	sem	x	sem				
Protein conc. (mg/g)	M	219.4	21.5	233.8	11.3	258.1	14.1	25.1	8.6	NS	NS	NS	NS
	LI	137.9	3.0	138.5	4.0	141.2	4.8	146.8	5.3	NS	NS	NS	NS
Protein /organ (mg)	M	19.8	2.7	22.2	2.8	16.4	1.6	13.4	1.5	NS	NS	NS	*
	LI	207.3	12.3	195.8	13.2	150.6	5.3	123.3	9.5	NS	NS (*)	**	**
[RNA] (μg/mg prot)	M	6.83	1.11	5.13	0.49	5.33	0.40	5.35	0.39	NS	NS	NS	NS
	LI	51.6	1.1	61.0	2.1	49.4	3.4	58.0	3.0	**	NS	NS	NS
[DNA] (μg/mg prot)	M	0.92	0.15	0.80	0.06	0.63	0.07	0.83	0.08	NS	NS	NS	NS
	LI	5.98	0.73	6.61	1.16	3.77	1.20	5.00	0.61	NS	NS	NS	NS

b)

		Saline (14)		T_4 (16)		High (14)		Low (16)		Test of significance	
		x	sem	x	sem	x	sem	x	sem	S v T_4	H v L
Protein conc. (mg/g)	M	238.7	13.5	228.9	6.8	226.6	11.8	239.5	8.6	NS	NS
	LI	139.5	2.8	143.2	3.5	138.2	2.4	144.3	3.6	NS	NS
Protein /organ (mg)	M	18.1	1.6	17.3	1.8	21.0	1.9	14.7	1.1	NS	**
	LI	178.9	10.2	155.0	12.0	201.5	8.8	135.2	6.7	NS(*)	***
[RNA] (μg/mg prot)	M	6.14	0.64	5.25	0.30	5.98	0.63	5.34	0.28	NS	NS
	LI	50.5	1.7	59.3	1.9	56.3	1.7	54.3	2.4	**	NS
[DNA] (μg/mg prot)	M	0.78	0.09	0.82	0.05	0.86	0.08	0.74	0.06	NS	NS
	LI	4.87	0.74	5.70	0.62	6.29	0.66	4.46	0.62	NS	*

Table 5.3.3

Rates of protein synthesis, degradation and growth and RNA activity in appetite selected mice either thyroidectomized of given a replacement dose of T_4.
M = gastrocnemius muscle and LI = liver.
Statistical test of significance: NS = not statistically shown to differ.
Parentheses indicate the number of animals per group.

Table 5.3.3
a)

		High				Low				Test of significance			
		Saline (7)		T_4 (7)		Saline (7)		T_4 (9)		HS v HT	LS v LT	HS v LS	HT v LT
		x	sem	x	sem	x	sem	x	sem				
FSR (%/d)	M	39.0	4.6	28.4	6.0	35.7	11.7	32.9	6.0	NS	NS	NS	NS
	LI	118.7	21.4	155.7	14.8	119.7	16.9	132.6	11.1	NS	NS	NS	NS
RNA activ. (FSR/ [RNA])	M	6.89	1.68	5.56	0.84	4.48	0.51	6.08	0.87	NS	NS	NS	NS
	LI	2.35	0.47	2.57	0.27	2.47	0.39	2.31	0.19	NS	NS	NS	NS
Prot. growth (mg/d)	M	0.84	0.86	0.63	1.05	0.21	1.21	0.19	1.17	NS	NS	NS	NS
	LI	6.59	5.75	8.58	7.03	3.17	8.12	1.20	7.86	NS	NS	NS	NS
FDR (%/d)	M	34.7	4.8	25.6	6.2	34.4	11.9	31.5	6.6	NS	NS	NS	NS
	LI	115.5	21.4	151.3	14.8	117.6	17.0	131.6	11.3	NS	NS	NS	NS

b)

		Saline (14)		T_4 (16)		High (14)		Low (16)		Test of significance	
		x	sem	x	sem	x	sem	x	sem	S v T_4	H v L
FSR (%/d)	M	46.5	10.5	31.0	4.2	33.7	3.9	42.2	9.7	NS	NS
	LI	119.2	13.1	142.7	9.2	137.2	13.5	127.0	9.5	NS	NS
RNA activity (FSR/ [RNA])	M	8.29	2.58	5.85	0.60	6.23	0.92	7.61	2.18	NS	NS
	LI	2.41	0.29	2.42	0.16	2.46	0.26	2.38	0.19	NS	NS

cases (Table 5.3.3).

Food Intake, Weight Gain and Food Conversion Efficiency.

Daily food intake did not differ between treatments but was significantly greater in H than L line animals ($p<0.001$) and this difference was maintained when differences in metabolic body size were taken into account (HS v LS, $p<0.05$; HT v LT, H v L, $p<0.01$, Table 5.3.4). Therefore, the direct response to selection was still evident under conditions altered from those of the original selection environment. No change was found between treatments in daily weight gain either corrected or uncorrected for differences in metabolic body size but there was a significant difference between lines (H>L; HS v LS, $p<0.05$; HT v LT, $p<0.01$; H v L, $p<0.001$, Table 5.3.4). Food conversion efficiency was not shown to differ between treatments but the H line tended to be more efficient than the L line (Table 5.3.4). This agrees with previous studies on these mice (Bishop and Hill, 1985). When the rate of protein synthesis was related to food intake it was lower in the H appetite groups for both skeletal muscle and liver: this comparison was made since protein synthesis is known to be significantly affected by nutrition, (Garlick et al, 1975; Millward et al, 1973). A similar pattern was observed when the rate of protein synthesis was related to weight gain.

Table 5.3.4

Food intake, weight gain, efficiency and rate of protein syntheis per unit food intake or weight gain in mice selected for appetite either thyroidectomized or given a replacement T_4 dose.
M = gastrocnemius muscle and LI = liver.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.
Parentheses indicate the number of animals per group.

Table 5.3.4
a)

	High				Low				Test of significance			
	Saline (7)		T_4 (7)		Saline (7)		T_4 (9)		HS v HT	LS v LT	HS v LS	HT v LT
	x	sem	x	sem	x	sem	x	sem				
FI/d (g/d)	3.72	0.13	4.11	0.20	2.34	0.12	2.31	0.11	NS	NS	***	***
FI/d.bwt$^{.75}$	.383	.015	.413	.008	.302	.017	.315	.016	NS	NS	*	**
Wt g/d (g/d)	.789	.029	.689	.086	.317	.065	.249	.047	NS	NS	**	**
Wt g/d.bwt$^{.75}$	.079	.008	.068	.008	.041	.008	.032	.006	NS	NS	*	**
Efficiency (Wt g/FI)	.213	.029	.165	.020	.132	.026	.108	.021	NS	NS	NS	NS
FSR / FI/d (%/g) M	10.7	1.5	7.2	1.7	23.0	8.4	14.9	3.1	NS	NS	NS	*
FSR / FI/d (%/g) LI	31.8	5.4	38.3	3.9	52.4	8.7	57.2	3.3	NS	NS	*	**
FSR / wt g/d (%/d) M	52.2	7.4	48.0	12.3	138.8	64.9	144.4	43.8	NS	NS	NS	*
FSR / wt g/d (%/d) LI	160.3	28.7	292.4	94.6	622.7	220.3	610.1	130.1	*	NS	*	*

b)

	Saline (14)		T_4 (16)		High (14)		Low (16)		Test of significance: S v T_4	Test of significance: H v L
	x	sem	x	sem	x	sem	x	sem		
FI/d (g/d)	3.03	0.21	3.11	0.25	3.91	0.13	2.36	0.08	NS	***
FI/d.bwt$^{.75}$	0.343	0.018	0.364	0.024	0.404	0.022	0.311	0.015	NS	**
Wt g/d (g/d)	0.577	0.083	0.441	0.072	0.739	0.064	0.279	0.038	NS	***
Wt g/d.bwt$^{.75}$	0.061	0.008	0.051	0.007	0.076	0.007	0.038	0.005	NS	***
Efficiency (Wt g/FI)	0.173	0.022	0.133	0.016	0.189	0.018	0.119	0.016	NS	*
FSR / FI/d (%/g) M	17.6	5.3	11.1	2.1	8.3	1.1	19.1	4.8	NS	*
FSR / FI/d (%/g) LI	41.0	4.4	50.0	4.6	35.0	3.2	55.2	4.2	NS	**
FSR / wt g/d (%/d) M	131.7	37.7	99.0	26.5	47.7	4.7	177.9	37.0	NS	***
FSR / wt g/d (%/d) LI	409.9	168.9	443.0	87.2	219.1	42.3	621.1	158.9	NS	***

5.4 DISCUSSION

In this study whole body growth did not respond to T_4 treatment. This is in contrast to data from Snell dwarf mice (dw/dw) and thyroidectomized rats which have much reduced growth rates compared with normal littermates and control rats respectively. This reduction in growth rate is reversible by treatment with T_4 (Holder and Wallis, 1977; Brown et al, 1981; Brown and Millward, 1983; Bates and Holder, 1986). The reason for the lack of response of body growth to T_4 treatment is not apparent but it is possible that the multiple injection procedure may have caused trauma in the mice and affected growth rates and appetite. Untreated controls would have helped to solve this problem but as explained previously lack of animals prevented this. There was no change in mean muscle weight or liver weight between treatments following the pattern of whole body growth. This is similar to previous studies. Bates and Holder (1986) recorded that upon T_4-treatment of Snell dwarf mice, even though whole body growth was increased, liver and muscle weights remained constant whereas heart growth increased. In addition, it was noted that daily T_4 injections to hypophysectomized rats caused an increase in body weight with an elevated heart/body weight ratio suggesting that T_4 may selectively stimulate heart growth (Gaspard et al, 1975). Brown et al (1981) found that even though T_3 treatment of thyroidectomized rats increased muscle growth rate above that of placebo treated counterparts, the growth rate was not restored to the level of control animals. With hindsight it may have been interesting to measure and compare heart weights.

Muscle protein synthesis rates did not alter significantly but were, if anything, slightly decreased on T_4 treatment. In previous studies thyroid hormone treatment greatly elevated the rate of muscle protein synthesis (Brown et al, 1981; Bates and Holder, 1986). It is notable that the protein synthesis rates were extremely high in this study although this seems to be a feature of replicate 6 since very high skeletal muscle turnover rates were evident in the first experiment using this replicate (Chap 3). These results infer that thyroid hormones have no effect on the rate of protein synthesis in skeletal muscle because T_4 did not stimulate protein turnover processes in either H or L appetite mice. As in the last chapter, it must be assumed that TBG levels remained unchanged between lines and that total T_4 concentrations reflected free T_4 concentrations. In comparison with experiment one (Chap 3), however, where the rate of protein synthesis in skeletal muscle of intact H and L line animals was shown to differ it appears that elimination of thyroid hormones has made this difference less clear. Therefore, different circulating levels of thyroid hormones may be implicated in the regulation of protein turnover in skeletal muscle of H and L appetite selected mice but their role remains equivocal. It is more probable that T_4 does not affect protein turnover in skeletal muscle of these mice.

In the liver since there was a tendency for a reduction in FSR upon thyroidectomy this may indicate a regulatory role for thyroid hormones in liver protein turnover. However, since both T_4 replacement in this study and data from Chapter 3 did not indicate any line differences in liver FSR then it does not appear that thyroid hormones differentially affect H and L groups. An

examination of the individual replicate FSR values (Appendix Table 3.3.3A) shows that in replicate 6, H line FSR was approximately 15% greater than L line FSR (although this difference was not significant). In the present study T_4 replacement tended to raise FSR levels in H appetite animals to a greater extent than in L mice. Thus, there is a hint that rates of protein synthesis in the liver of H mice may be more responsive to thyroid hormones. However, because thyroid hormones are naturally present in lower concentrations in the H appetite line (Chapter 4), this difference would not normally be revealed in intact mice.

No difference was found in either skeletal muscle RNA concentration or RNA activity between treatments which reinforces the lack of effect of T_4 on skeletal muscle protein synthesis rates. The difference in liver RNA concentrations between treatments indicates that T_4 may have a regulatory effect on liver protein synthesis processes although differences in liver protein synthesis rates only tend towards significance. Previous studies in which thyroid hormones have been found to stimulate skeletal muscle protein synthesis rates (Brown et al, 1981; Brown and Millward, 1983; Brown et al, 1983; Bates and Holder, 1986) indicate that this is achieved by raising RNA capacity (Brown et al, 1981; Brown and Millward, 1983; Brown et al, 1983; Flaim et al, 1978) although in two cases an increase in RNA activity was also recorded (Brown and Millward, 1983; Bates and Holder, 1986). Increases in the rate of liver protein synthesis appear to be induced by both increases in RNA capacity and RNA activity (Brown et al, 1983; Bates and Holder, 1986).

The rates of protein degradation in both liver and skeletal muscle reflected the rates of protein synthesis. Thyroxine treatment therefore tended to increase protein degradation rates in the liver to a greater extent in H than L line mice. In other studies T_4 treatment has been shown to increase rates of protein degradation both in liver and muscle (De Martino and Goldberg, 1978; Goldberg, 1980; Brown et al, 1981; Hayashi, Kayali and Young, 1986). On the other hand, it is interesting that in thyroid hormone-induced cardiac hypertrophy the rate of protein synthesis was raised but unlike muscle and liver the rate of protein degradation was slightly decreased (Sanford et al, 1978), indicating that thyroid hormones can act differently in various tissues.

In this study upon standardization of thyroid hormone levels the food intake per unit metabolic body size was still considerably greater in the H line than the L line. Thus, thyroid hormones do not seem to have a fundamental influence on appetite regulation in these mice. In nutritional studies it is well documented that food intake is positively correlated with thyroid hormone concentrations (Cox, Dalal, Heard and Millward, 1984; Ortiz-Caro et al, 1984; Ryg, 1984). Selection, appears to have broken this relationship possibly exploiting the natural variance between these two parameters. This relationship can also be manipulated by other factors eg seasonal changes in fat deposition in reindeer make the association less pronounced (Ryg, 1984).

The increased food conversion efficiency between comparable H and L groups agrees with previous studies on these mice (Bishop and Hill, 1984). However, this may not represent a real increase in the

efficiency of the H line but may only be the result of a larger food intake per unit metabolic body weight. Consequently less energy is spent on maintenance per unit weight gain and therefore, the efficiency increases. However, this is only a reflection of whole body efficiency and gives no information on the efficiency of gain for different tissues or organs. To gain an impression of the relative efficiency of protein accretion in skeletal muscle and liver the rates of protein synthesis for these different organs were related to weight gain and food intake. Ideally the rates of protein synthesis for a particular organ would be examined alongside the protein increment for that organ but obviously since invasive techniques would be necessary this was impossible. In skeletal muscle there were no treatment differences in FSR proportional to food intake or weight gain which implies that thyroid hormones do not influence the efficiency of skeletal muscle accretion. There was a significant line difference; the rate of protein synthesis per unit weight gain or per unit food intake in the L line was approximately double that of H line counterparts which implies that differences in rates of protein turnover may contribute to differences in food conversion efficiency.

In the liver, rates of protein synthesis per unit food intake or weight gain were also higher in L line groups. In Chapter 3 it was shown that the rate of protein synthesis in liver of intact H appetite mice was similar or if anything slightly greater than L line mice suggesting that liver protein growth in the H line may have been as efficient or a little less efficient than in the L line. However, the analysis of protein synthesis rate in relation to weight gain or food intake in this experiment has reversed this

tentative conclusion and emphasizes that protein turnover rates in isolation cannot give a true idea of efficiency. Therefore, despite protein turnover perhaps being higher in H line liver, liver tissue was nevertheless laid down more efficiently in H appetite groups. Moreover, since whole body maintenance requirements are higher in H line mice (Bishop and Hill, 1985) this strengthens the inference that another process must have much higher energy consumption rates in H line animals.

Relating protein synthesis to weight gain assumes that whole body weight gain is proportional to organ and tissue gain which may not be entirely correct and so these figures must be viewed with caution. However, taking both the rate of protein synthesis in relation to weight gain and to food intake should yield a certain amount of useful information.

This study has indicated that thyroid hormones do not appear to be the major cause of appetite or correlated FSR differences in mice selected for food intake if total thyroid hormone concentrations reflected free thyroid hormone concentrations.

CHAPTER SIX

GENERAL DISCUSSION

6.1 INTRODUCTION

The present study has established and begun to investigate in detail some metabolic differences between mice divergently selected for food intake. The long term objective of the research programme was to characterize metabolic traits that may be useful as selection criteria in a breeding programme. Protein turnover was the trait concentrated upon in this study given that there was a good case for differences in protein turnover being linked with a substantial proportion of the divergence in growth rate and food intake evident between the groups of mice. In this discussion the major findings will be summarized and their influence on whole body energy balance and growth status discussed.

6.2 RATES OF PROTEIN TURNOVER

The major finding in the first experiment (Chapter 3) was that the rate of protein synthesis was significantly lower in skeletal muscles of H than L appetite mice. It is notable that Priestley and Robertson (1973) found a similar alteration in the rate of protein turnover in livers and kidneys of mice selected for high and low six week body weight. Likewise, comparison of the rate of muscle protein metabolism between a rapidly growing and a slow growing strain of rat also showed a similar difference (Millward *et al*, 1975; Bates and Millward, 1978). Usually within a particular strain there is a direct positive correlation between growth rate and protein turnover rate (Millward *et al*, 1975). Thus, inter- and intrastrain variation in this parameter do not follow the same pattern.

With respect to maintenance energy requirements and efficiency what does this change in the rate of protein turnover signify? In skeletal muscle it appears that maintenance energy requirements are lower in the H line and this group has a greater efficiency of protein deposition. The impact of these alterations upon whole body protein turnover can be roughly estimated. Skeletal muscle contributes approximately 20% to whole body protein turnover (Garlick, 1980) (the figure is up to double this in farm species) and the difference in the rates of protein synthesis between H and L lines was approximately 20% (15.2 v 18.2%/d). This represents about a 4% alteration in turnover of whole body protein in favour of the H line.

Bishop and Hill (1985) demonstrated that requirements for whole body maintenance were greater in H appetite mice than L group counterparts. Thus, despite skeletal muscle protein turnover in the H line having lower maintenance requirements, the situation for the whole body is the converse. It can only be concluded that either other proteinaceous tissues (not liver or small intestine) have a greater basal turnover rate in the H line or another metabolic process is the cause of these elevated maintenance requirements. Therefore, differential alterations in maintenance needs appear to have occurred in different organs/tissues with appetite selection. It would be advantageous to pinpoint precisely where extra maintenance energy is being utilized and then select animals for the benefit of lower protein turnover rate in skeletal muscle with a higher growth rate but without the disadvantage of the other energetically more expensive process(es) also being chosen.

6.3 THYROID HORMONE INVOLVEMENT

A difference in plasma T_4 concentration was discovered between lines. T_4 levels tended to be lower in the H appetite mice. Upon manipulation of T_4 the findings were equivocal and thyroid hormones may or may not be involved in the control of both skeletal muscle and liver protein turnover in these mice.

There was not a positive correlation between thyroid hormone levels and food intake in the second experiment (Chapter 4) when plasma thyroid hormone concentrations were determined. Upon thyroid hormone manipulation when hormones concentrations were standardized (Chapter 5) the lines still differed in the amount of food they ate. Hence, thyroid hormones do not seem to be the primary factor that caused the appetite differences in these mice.

Therefore, the questions remain: what effect does the difference in thyroid hormone levels have upon these mice? What causes the difference in the rate of skeletal muscle protein turnover? Which aspect of metabolism is so much greater in the H than L line so as to negate the greater efficiency of skeletal muscle protein turnover?

The first point to establish in further studies would be whether there were any differences in TBG levels between lines in order that tT_4 concentrations could be equated with fT_4 concentrations. Also, it would be interesting to find what the difference in skeletal muscle protein turnover was due to. Other hormones such as GH and insulin could be investigated in the same manner as thyroid hormones

were approached. However, it must be accepted with this type of approach that it may be difficult to detect differences since many hormones may be involved, each having a small effect.

Since the difference in requirements for maintenance energy do not appear to be due to differences in protein turnover, another metabolic process must be involved. Brown adipose tissue may provide the answer and may be a good area on which to concentrate further research. It would be reasonable to first quantify the amount of brown adipose tissue in the lines to see if it differed. A parallel study to look at the activity of the tissue should also be performed since amounts of tissue alone are useless. The physical activity of the mice may also be important and worth monitoring although through working with these animals such differences were not apparent. Alternatively, the differences in energy expenditure may be due to H line mice having a leaky sodium pump which could dissipate large amounts of energy.

6.4 SUMMARY

i) The FSR and by implication FDR in skeletal muscle of H line mice was lower than in L line mice. No clear corresponding differences were found in either liver or small intestine.

ii) Thyroid hormone concentrations varied in a way that may have indicated they were involved in control of skeletal muscle synthesis and degradation rates.

iii) However, manipulation of thyroid hormone concentrations did not support a major role for thyroid hormones in regulating skeletal muscle or liver FSR/FDR.

iv) The greater apparent skeletal muscle protein efficiency of H lines does not match up with whole body maintenance requirements (Bishop and Hill, 1985). Therefore, some other processes must be less efficient in H than L lines.

v) Skeletal muscle FSR and thyroid hormone concentrations vary genetically in a way that is distinct from changes due to nutrition.

APPENDIX

Table 3.3.1A Body and organ weights of mice divergently selected for 4–6 week food intake (individual replicates).

		High		Control		Low		Test of significance		
	Rep	x	sem	x	sem	x	sem	H–L	H–C	C–L
Bodyweight (g)	1	30.7	1.0	26.9	0.9	24.7	0.6	**	*	*
	2	29.7	0.8	–	–	22.8	0.7	**	–	–
	3	30.7	0.1	26.0	0.1	24.0	0.1	**	**	**
	6	28.2	1.4	–	–	21.0	0.9	**	–	–
Mean muscle weight (g)	1	.125	.007	.115	.006	.118	.004	NS	NS	NS
	2	.149	.006	–	–	.104	.004	**	–	–
	3	.137	.006	.105	.005	.109	.005	**	**	NS
	6	.138	.010	–	–	.094	.026	**	–	–
Liver weight (g)	1	1.973	0.049	1.756	0.083	1.485	0.038	**	NS	*
	3	1.999	0.071	1.816	0.067	1.449	0.029	**	NS	**
	6	1.615	0.075	–	–	1.275	0.066	*	–	–
Small intestine weight (g)	1	1.395	0.051	1.340	0.064	1.034	0.055	**	NS	**
	3	1.565	0.083	1.372	0.073	1.042	0.053	**	NS	**
	6	1.328	0.074	–	–	0.931	0.021	**	–	–

Statistical test of significance: NS = not statistically shown to differ, * = $p<0.05$ and ** = $p<0.01$.

Table 3.3.2A Gastrocnemius muscle, liver and small intestine protein, RNA and DNA concentrations and total organ protein contents of mice divergently selected for appetite (individual replicates).

			High		Control		Low		Test of significance		
		Rep	x	sem	x	sem	x	sem	H–L	H–C	C–L
Protein conc (mg/g wet wt)	M	1	178.9	8.4	187.7	9.7	189.7	14.1	NS	NS	NS
		2	169.1	11.1	–	–	196.1	11.8	NS	–	–
		3	231.5	28.4	171.2	15.1	193.1	25.7	NS	NS	NS
		6	180.1	17.5	–	–	181.6	13.6	NS	–	–
	LI	1	128.3	6.1	139.9	7.5	125.5	5.5	NS	NS	NS
		3	169.8	15.3	202.8	21.7	195.7	14.9	NS	NS	NS
		6	152.2	6.4	–	–	131.5	4.9	*	–	–
	SI	1	118.5	7.4	113.6	9.8	83.4	10.3	**	NS	*
		3	135.7	10.3	118.5	6.8	134.1	10.9	NS	NS	NS
		6	122.3	3.3	–	–	123.0	3.9	NS	–	–
RNA conc (μg/mg prot)	M	1	9.45	0.64	8.36	0.57	7.68	0.57	*	NS	NS
		2	6.31	0.89	–	–	6.55	1.20	NS	–	–
		3	7.24	2.05	7.80	0.57	8.16	2.49	NS	NS	NS
		6	8.90	0.71	–	–	10.79	0.92	NS(*)	–	–
	LI	1	72.1	3.2	63.3	3.0	67.0	3.4	NS	*	NS
		3	58.7	4.9	50.3	5.6	52.8	7.0	NS	NS	NS
		6	55.4	2.8	–	–	59.9	2.0	NS	–	–
	SI	1	100.2	5.8	116.1	7.6	156.1	15.9	**	NS	*
		3	61.9	6.9	82.6	7.8	64.4	4.4	NS	*	NS
		6	86.1	3.9	–	–	75.1	1.8	**	–	–
DNA conc (μg/mg prot) conc	M	1	8.75	0.55	6.70	0.49	6.62	0.58	*	**	NS
		2	2.47	0.35	–	–	2.98	0.30	NS	–	–
		6	1.44	0.08	–	–	1.72	0.14	NS	–	–
	LI	1	27.2	2.9	29.6	2.5	26.1	2.9	NS	NS	NS
		3	19.2	1.4	15.5	1.7	15.2	1.1	NS	NS	NS
		6	7.0	0.8	–	–	8.6	0.8	NS	–	–
	SI	1	181.2	12.8	186.3	13.7	239.7	17.3	**	NS	*
		3	55.2	4.3	62.2	3.6	64.7	7.1	NS	NS	NS
		6	55.0	4.7	–	–	54.3	4.4	NS	–	–
Protein content per organ (mg)	M	1	22.4	1.7	21.3	1.4	22.2	1.6	NS	NS	NS
		2	24.0	2.1	–	–	19.9	1.6	NS	–	–
		3	30.7	2.9	18.2	1.9	20.8	2.6	*	**	NS
		6	24.7	2.8	–	–	17.0	1.7	*	–	–
	LI	1	254.4	15.6	249.2	20.1	185.7	8.5	**	NS	**
		3	341.1	35.6	364.2	36.2	284.2	24.2	*	**	NS
		6	244.6	13.9	–	–	168.4	11.7	**	–	–
	SI	1	166.9	13.9	154.1	17.1	86.0	11.3	**	NS	**
		3	212.1	19.4	163.2	14.1	138.2	10.5	**	NS	NS
		6	162.2	8.9	–	–	14.1	2.9	**	–	–

M = gastrocnemius muscle, LI = liver and SI = small intestine.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$ and ** = $p<0.01$.

Table 3.3.3A Rates of protein synthesis and RNA activities of various tissues from mice selected for 4-6 week food intake (individual replicates).

			High		Control		Low		Test of significance		
		Rep	x	sem	x	sem	x	sem	H-L	H-C	C-L
FSR (%/d)	M	1	17.0	1.2	13.9	1.2	14.0	0.9	NS	*	NS
		2	17.0	2.9	-	-	25.0	3.0	*	-	-
		3	9.9	0.9	10.9	1.0	12.9	1.3	NS(*)	NS	NS
		6	20.1	2.4	-	-	31.1	5.0	*	-	-
	LI	1	83.7	6.0	107.1	6.2	97.4	6.0	NS	*	NS
		3	92.1	3.9	99.3	5.1	78.8	6.5	NS(*)	NS	*
		6	143.4	11.1	-	-	124.4	10.7	NS	-	-
	SI	1	73.9	3.8	81.9	5.3	82.6	4.7	NS	NS	NS
		3	82.4	4.3	78.5	3.9	72.6	5.5	NS(*)	NS	NS
		6	88.3	0.1	-	-	106.9	6.8	NS	-	-
RNA activity (RNA/[FSR])	M	1	1.91	0.18	1.76	0.20	2.01	0.18	NS	NS	NS
		2	3.44	1.08	-	-	5.11	1.05	NS	-	-
		3	2.28	0.59	1.41	0.09	2.69	1.00	NS	NS	NS
		6	2.33	0.31	-	-	2.40	0.17	NS	-	-
	LI	1	1.19	0.10	1.71	0.10	1.49	0.09	NS	**	NS
		3	1.64	0.14	2.16	0.27	1.67	0.27	NS	NS	NS
		6	2.61	0.20	-	-	2.10	0.18	NS(*)	-	-
	SI	1	0.76	0.05	0.74	0.06	0.63	0.10	*	NS	NS
		3	1.47	0.17	0.99	0.10	1.18	0.13	NS	*	NS
		6	1.03	0.07	-	-	1.42	0.07	**	-	-

M = gastrocnemius muscle, LI = liver and SI = small intestine.
Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$ and ** = $p<0.01$.

Table 4.3.1A Body weights and plasma thyroid hormone concentrations at five weeks of age in mice selected for appetite (individual replicates).

		High		Control		Low		Test of significance		
		x	sem	x	sem	x	sem	H-L	H-C	C-L
Body weight (g)	1	26.7	1.6	23.9	1.0	23.5	0.7	*	*	NS
	2	31.5	1.7	21.1	2.2	28.8	0.5	NS	**	**
	3	25.1	0.7	23.1	0.6	21.4	0.5	**	*	NS
tT_4 (ng/ml)	1	42.2	5.2	57.4	4.1	37.6	3.8	NS	NS(*)	**
	2	57.0	5.9	48.3	3.7	80.0	4.3	**	NS	**
	3	42.3	1.9	61.1	2.6	62.0	5.6	**	**	NS
tT_3 (ng/ml)	1	0.88	0.05	0.94	0.06	0.85	0.03	NS	NS	NS
	2	1.15	0.04	0.83	0.09	1.21	0.09	NS	*	*
	3	0.86	0.02	0.81	0.04	1.00	0.08	NS	NS	*

Statistical test of significance: NS = not statistically shown to differ, (*) = $p<0.1$, * = $p<0.05$ and ** = $p<0.01$.

Table 4.3.2A Body weights and plasma thyroid hormone concentrations at six weeks of age in appetite selected mice (individual replicates).

		High		Control		Low		Test of significance		
		x	sem	x	sem	x	sem	H-L	H-C	C-L
Body weight (g)	1	28.0	0.6	26.1	0.7	20.5	0.5	***	NS	***
	2	29.9	0.7	26.7	0.7	27.2	0.7	*	**	NS
	3	27.3	1.1	27.7	1.0	21.9	0.6	***	NS	***
	6	30.0	0.5	-	-	24.5	0.7	***	-	-
tT_4 (ng/ml)	1	47.0	1.8	43.8	2.0	46.2	2.1	NS	NS	NS
	2	57.4	2.2	59.2	3.0	56.4	2.8	NS	NS	NS
	3	39.6	1.6	59.3	2.0	45.4	1.8	*	***	***
	6	54.2	1.8	-	-	58.0	2.2	NS	-	-
tT_3 (ng/ml)	1	1.10	0.04	1.00	0.04	1.08	0.05	NS	NS	NS
	2	1.28	0.04	1.10	0.05	1.10	0.04	**	**	NS
	3	0.94	0.02	1.07	0.04	1.03	0.09	NS	**	NS
	6	1.20	0.03	-	-	2.46	0.10	***	-	-

Statistical test of significance: NS = not statistically shown to differ, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

BIBLIOGRAPHY

BIBLIOGRAPHY

ALBERTSE, E.C., GARLICK, P.J. & PAIN, V.M. (1979) Whole-body protein synthesis and oxidation rates in streptozotocin diabetic rats. Proc. Nutr. Soc. 38, 125A.

ALLEN, P. & McCARTHY, J.C. (1980) The effects of selection for high and low body weight on the proportion and distribution of fat in mice. Anim. Prod. 31, 1-11.

ANGERAS, U. & HASSELGREN, P-O. (1985) Protein turnover in different types of skeletal muscle during experimental hyperthyroidism in rats. Acta endocr., Copnh. 109, 90-95.

ANGERAS, U. & HASSELGREN, P-O. (1987) Protein degradation in skeletal muscle during experimental hyperthyroidism in rats and the effect of β-blocking agents. Endocrinology 120(4), 1417-1421.

ARNAL, M., FERRARA, M. & FAUCONNEAU, G. (1976) in Nuclear Techniques in Animal Production and Health, pp 393-401, International Atomic Energy Agency, Vienna.

AZIZI, F. (1978) Effect of dietary composition on fasting induced changes in serum thyroid hormones and thyrotropin. Metabolism 27, 935-942.

BACHMAIR, A., FINLEY, D. & VARSHAVKY, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. Science, N.Y. 234, 179-186.

BAIRD, D.M., NALBANDOV, A.V. & NORTON, H.W. (1952) Some physiological causes of genetically different rates of growth in swine. J. Anim. Sci. 11, 292-300.

BAKER, R.L. & CHAPMAN, A.B. (1975) Correlated responses to selection for postweaning gain in the rat. Genetics, Princeton. 80, 191-203.

BAKER, R.L., CARTER, A.H. & COX, E.H. (1979) The effect of selection for body weight at different ages on fat deposition in mice. Proc. N.Z. Soc. Anim. Prod. 39, 118-128.

BARRIA, N. & BRADFORD, G.E. (1981) Long-term selection for rapid gain in mice. I Genetic analysis at the limit of response. J. Anim. Sci. 52(4), 729-738.

BARTKE, A. (1965) The response of two types of dwarf mice to growth hormone, thyrotropin and thyroxine. Gen. comp. Endocr. 5, 418-426.

BATES, P.C. & MILLWARD, D.J. (1978) Muscle growth and protein turnover in a fast growing rat strain. Proc. Nutr. Soc. 37, 19A.

BATES, P.C. & MILLWARD, D.J. (1981) Characteristics of skeletal muscle growth and protein turnover in a fast-growing rat strain. Br. J. Nutr. 46, 7-13.

BATES, P.C. & HOLDER, A.T. (1986) The anabolic effects of growth hormone and thyroxine on tissue growth in Snell dwarf mice. Proc. Nutr. Soc. 46, 35A.

BERNIER, J.F., CALVERT, C.C., FAMULA, T.R. & BALDWIN, R.L. (1986) Maintenance energy requirement and net energetic efficiency in mice with a major gene for rapid postweaning gain. J. Nutr. 116, 419-428.

BEST, J.M.J. (1972) The use of trienbolone acetate implants in heifer beef production. Vet. Rec. 91, 624-626.

BIONDINI, P.E., SUTHERLAND, T.M. & HAVERLAND, L.H. (1968) Body composition of mice selected for rapid growth rate. J. Anim. Sci. 27, 5-12.

BISHOP, S.C. & HILL, W.G. (1985) Effects of selection on growth, body compsition, and food intake in mice. III. Correlated responses: growth, body composition, food intake and efficiency and catabolism. Genet. Res. 46, 57-74.

BLAXTER, K.L. (1968) in Growth and Development of Animals (Lodge, G.A. & Lamming, G.E., eds.), pp329-344, Butterworths, London.

BLYTH, C.A., COOPER, M.B., ROOBOL, A. & RATIN, B.R. (1972) The binding of steroid hormones to degranulated microsomes from rat-liver endoplasmic reticulum. Eur. J. Biochem. 29, 293-300.

BOCKLEN, E., FLAD, S., MULLER, E. & von FABER, H. (1986) Comparative determination of beta-adrenergic receptors in muscle, heart and backfat of pietrain and large white pigs. Anim. Prod. 43, 335-340.

BOER, G.J. (1975) A simplified microassay of DNA and RNA using ethidium bromide. Analyt. Biochem. 65, 225-231.

BOWMAN, P. & McLAREN, A. (1970) Cell numbers in early embryos from strains of mice selected for large and small body size. Genet. Res. 15, 261-263.

BRADFORD, G.E. & FAMULA, T.R. (1984) Evidence for a major gene for rapid postweaning growth in mice. Genet. Res. 44, 293-308.

BRODY, S. (1945) Bioenergetics and Growth with special reference to the efficiency complex in domestic animals. Reinhold, New York.

BROWN, J.G. & MILLWARD, D.J. (1983) Dose response of protein turnover in rat skeletal muscle to triiodothyronine treatment. Biochim. biophys. Acta 757, 182-190.

BROWN, J.G., BATES, P.C., HOLLIDAY, M.A. & MILLWARD, D.J. (1981) Thyroid hormones and muscle protein turnover. Biochem. J. 194, 771-782.

BROWN, J.G., van BUENEN, J. & MILLWARD, D.J. (1983) The effect of triiodothyronine administration on protein synthesis in the diabetic rat. Biochem. J. 214, 637-640.

BROWN, M.A. & FRAHM, R.R. (1975) Feed efficiency in mice selected for preweaning and postweaning growth. J. Anim. Sci. 41(4), 1002-1007.

BURKE, W.H. & MARKS, H.L. (1982) Growth hormone and prolactin levels in non-selected and selected broiler lines of chickens from hatch to eight weeks of age. Growth 46, 283-295.

BURNS, A.H. & REDDY, W.J. (1975) Direct effect of thyroid hormones on glucose oxidation by isolated rat cardiac myocytes. J. Mol. Cell. Cardiol. 7, 553-561.

BYRNE, I., HOOPER, J.C. & McCARTHY, J.C. (1973) Effects of selection for body size on the weight and cellular structure of seven mouse muscles. Anim. Prod. 17, 187-196.

CALVERT, C.C., FAMULA, T.R. & BRADFORD, G.E. (1984) Composition of growth in mice with a major gene for rapid postweaning gain. J. Anim. Sci. 59, 361-365.

CALVERT, C.C., FAMULA, T.R., BERNIER, J.F. & BRADFORD, G.E. (1985) Serial composition during growth in mice with a major gene for rapid postweaning growth. Growth 49, 246-257.

CANOLTY, N.L. & KOONG, L.J. (1976) Utilization of energy for maintenance and for fat and lean gains by mice selected for rapid postweaning growth rate. J. Nutr. 106, 1202-1208.

CARTER, W.J., FAAS, F.H. & WYNN, J.O. (1975) Demonstration of thyroxine-stimulated incorporation of amino acid into peptide linkage in mitochondria-free system. J. biol. Chem. 250(10), 3588-3594.

CARTER, W.J., FAAS, F.H. & WYNN, J.O. (1976) Stimulation of peptide elongation by thyroxine. Biochem J. 156, 713-717.

CARTER, W.J., BENJAMIN, W.S.W. & FAAS, F.H. (1980) Effect of experimental hypothyroidism on protein turnover in skeletal and cardiac muscle. Metabolism 29(10), 910-915.

CARTER, W.J., BENJAMIN, W.S.W. & FAAS, F.H. (1982) Effect of experimental hyperthyroidism on protein turnover in skeletal and cardiac muscle as measured by [^{14}C] tyrosine infusion. Biochem. J. 204, 69-74.

CHAI, C.K. (1970) Selective breeding for thyroid $^{[131]}$I uptake in mice. Genetics, Princeton 64, 29-40.

CHAN, K.H., HEITZMAN, R.J. & KITCHENHAM, B.A. (1975) Digestability and N-balance studies on growing heifers implanted with trienbolone acetate. Br. vet. J. 131, 170-174.

CHEUNG, T.K. & PARKER, R.J. (1974) Effect of selection on heritability and genetic correlation of two quantitative traits in mice. Can. J. Genet. Cytol. 16, 599-609.

CHIN, R.C. & KIDSON, C. (1971) Selective associations of hormonal steroids with aminoacyl transfer RNAs and control of protein synthesis. Proc. natn. Acad. Sci. USA. 68, 2448.

CHIRASEVEENUPRAPUND, S., BUERGI, V., GOSWAMI, A. & ROSENBERG, I.N. (1975) in Thyroid Research, Proceedings of the Seventh International Thyroid Conference, (Robbins, J. & Braverman, L.E., eds.), pp244-247, American Elsevier Publishing Company, New York.

CHOPRA, I.J., SOLOMON, D.H., CHOPRA, U., WU, S.Y., FISHER, D.A. & NAKAMURA, Y. (1975) Pathways of metabolism of thyroid hormones. Rec. Prog. Horm. Res. 34, 521-567.

COX, M.D., DALAL, S.S., HEARD, C.R.C. & MILLWARD, D.J. (1984) Metabolic rate and thyroid status in rats fed diets of different

protein-energy value: The importance of free triiodothyronine. J. Nutr. 114(9), 1609-1616.

CROALL, D.E. & de MARTINO, G.N. (1983) Purification and characterization of calcium-dependent proteases from rat heart. J. biol. Chem. 258, 5660-5665.

CZECH, M.P., MALBON, C.C., KERMAN, K., GITOMER, W. & PILCH, P.F. (1980) Effect of thyroid status on insulin action in rat adipocytes and skeletal muscle. J. Clin. Invest. 66, 574-582.

DALTON, D.C. (1967) Selection for growth in mice on two diets. Anim. Prod. 9, 425-434.

de MARTINO, G. & GOLDBERG, A.L. (1978) Thyroid hormones control lysosomal enzyme activities in liver and skeletal muscle. Proc. natn. Acad. Sci. USA. 75, 1369-1373.

DEB, S., MARTIN, R.J. & HERSHBERGER, T.V. (1976) Maintenance requirements and energetic efficiency of lean and obese Zucker rats. J. Nutr. 106, 191-197.

DONALD, H.P., READ, J.L. & RUSSELL, W.S. (1970) Influence of litter size and breed of sire on carcass weight and quality of lambs. Anim. Prod. 12, 281-290.

EDMUNDS, B.K., BUTTERY, P.J. & FISHER, C. (1978) Protein turnover and whole-body nitrogen metabolism in the growing pig. Proc. Nutr. Soc. 37, 32A.

EDWARDS, R.G. (1962) The size and endocrine activity of the pituitary in mice selected for large or small size. Genet. Res. 3, 428-443.

EISEN, E.J. (1978) Single trait and antagonistic index selection for litter size and body weight in mice. Genetics, Princeton 88, 781-811.

EISEN, E.J. & BANDY, T. (1977) Correlated responses in growth and body composition of replicated single-trait and index selected lines of mice. Theor. Appl. Genet. 49, 133-144.

EISEN, E.J., LANG, B.J. & LEGATES, J.E. (1969) Comparison of growth functions within and between lines of mice selected for large and small body weight. Theor. Appl. Genet. 39, 251-260.

ENGELHARDT, W.V., DELLOW, D.W. & HOELLER, H. (1985) The potential of ruminants for the utilizaation of fibrous low-quality diets.Proc. Nutr. Soc. 44, 37-43.

ESBER, H.J., MENNINGER, F.F.,Jr. & BOGDEN, A.E. (1974) Variation in serum hormone concentrations in different rat strains. Proc. Soc. exp. Biol. Med. 146, 1050-1053.

FALCONER, D.S. (1953) Selection for large and small size in mice. J Genet. 51, 470-501.

FALCONER, D.S. (1960) Selection of mice for growth on high and low planes of nutrition. Genet. Res. 1, 91-113.

FALCONER, D.S. & LATYSZEWSKI, M. (1952) The environment in relation to selection for size in mice. J. Genet. 51, 67-80.

FITZHUGH, H.A.,Jr. (1976) Analysis of growth curves and strategies for altering their shape. J. Anim. Sci. 42, 1036-1051.

FLAIM, K.E., LI, J.B. & JEFFERSON, L.S. (1978) Effects of thyroxine on protein turnover in rat skeletal muscle. Am. J. Physiol. 235(2), E231-E236.

FLECK, A. & BEGG, D. (1965) The estimation of ribonucleic acid using ultraviolet absorption measurements. Biochim. biophys. Acta 108, 333-339.

FOSTER, D.O. & FRYDMAN, M.L. (1978) Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. Can. J. Physiol. & Pharmacol. 56, 110-122.

FOWLER, R.E. (1958) The growth and carcass composition of strains of mice selected for large and small body size. J. agric. Sci., Camb. 51, 137-149.

FOWLER, R.E. (1962) The efficiency of food utilization, digestability of foodstuffs and energy expenditure of mice selected for large or small size. Genet. Res. 3, 51-68.

FOWLER, V.R., BICHARD, M. & PEASE, A. (1976) Objectives in pig breeding. Anim. Prod. 23, 365-387.

FUNABIKI, R., WATANABE, Y., NISHIZAWA, N. & HAREYAMA, S. (1976) Quantitative aspect of the myofibrillar protein turnover in transient state on dietary protein depletion and repletion revealed by urinary excretion of N^t-methylhistidine. Biochim. biophys. Acta 451, 143-150.

GALBRAITH, H. & WATSON, H.B. (1978) Performance, blood and carcase characteristics of finishing steers treated with trenbolone acetate and hexoestrol. Vet. Rec. 103, 28-30.

GALL, G.A.E., KYLE, W.H., ROGLER, J.C. & ANDERSON, V.L. (1967) Genetic parameters of protein metabolism and growth in mice. Genetics 56, 215-225.

GALLO, G., VOCI, A., SCHWARZE, P.E. & FUGASSA, E. (1987) Effect of tri-iodothyronine on protein turnover in rat hepatocyte primary cultures. J. Endocr. 113, 173-177.

GARLICK, P.J. (1980) in Comprehensive Biochemistry Vol. 19B part 1, (Florkin, M., Neuberger, A. & Van Deenan, L.L.M., eds.), pp 77-152, Elsevier, Amsterdam.

GARLICK, P.J. (1980) in Protein Deposition in Animals (Buttery, P.J. & Lindsay, D.B., eds.), pp51-67, Butterworths, London.

GARLICK, P.J., MILLWARD, D.J., JAMES, W.P.T. & WATERLOW, J.C. (1975) The effect of protein deprivation and starvation on the rate of protein synthesis of the rat. Biochim. biophys. Acta 414, 71-84.

GARLICK, P.J., BURK, T.L. & SWICK, R.W. (1976) Protein synthesis and RNA in tissues of the pig. Am. J. Physiol. 230, E1108-E1112.

GARLICK, P.J., McNURLAN, M.A. & PREEDY, V.R. (1980) A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H] phenylalanine. Biochem. J. 192, 719-723.

GARLICK, P.J., FERN, M. & PREEDY, V.R. (1983) The effect of insulin and food intake on muscle protein synthesis in postabsorptive rats. Biochem. J. 210, 669-676.

GASPARD, S.T., WONDERGEM, R. & KLITGAARD, H.M. (1975) Effect of growth hormone and thyroxine on serum somatomedin and growth. Fedn.

Proc. 34, 307A.

GLASS, A.R., YOUNG, R.A. & ANDERSON, J. (1986) Decreased serum 3,5,3'-triiodothyronine (T_3) and abnormal serum binding of T_3 in calorie-deficient rats: adaptation after chronic underfeeding. Endocrinology 118(6), 2464-2469.

GODDARD, C., WILKIE, R. & DUNN, I.C. (1988) The relationship between insulin-like growth factor-I, growth hormone, thyroid hormones and insulin in chickens selected for growth. In press.

GOLDBERG, A.L. (1969) Protein turnover in skeletal muscle. I Protein catabolism during work-induced hypertrophy and growth induced with GH. J. biol Chem. 244, 3217-3222.

GOLDBERG, A.L. & DICE, J.F. (1974) Intracellular protein degradation in mammalian and bacterial cells. A. Rev. Biochem. 43, 835-869.

GOLDBERG, A.L., TISCHLER, M., de MARTINO, G. & GRIFFIN, G. (1980) Hormonal regulation of protein degradation and synthesis in skeletal muscle. Fedn. Proc. 39, 31-36.

GOLDSPINK, D.F. & KELLY, F.J. (1984) Protein turnover and growth in the whole body, liver and kidney of the rat from foetus to senility. Biochem. J. 217, 507-516.

GOLDSPINK, D.F., LEWIS, S.E.M. & KELLY, F.J. (1984) Protein synthesis during the developmental growth of the small and large intestine of the rat. Biochem. J. 217, 527-534.

GOODALE, H.D. (1938) A study of the inheritance of body weight in the Albino mouse by selection. J. Hered. 29, 101-112.

GRAHAM, A. & HOOPER, C. (1982) Selection for muscle fibre number in mice. Anim. Breed. Abstr. 50(1), 71A.

GRAHAM, A. & McCARTHY, J.C. (1982) Food consumption in large and small lines of mice. Anim. Breed. Abstr. 50, 69A.

GROSS, J. & PITT-RIVERS, R. (1952) Physiological activity of 3:5:3'-L-triiodothyronine. Lancet i, 593-594.

HANRAHAN, J.P., EISEN, E.J. & LEGATES, J.E. (1973a) Effects of population size and selection intensity on short-term response to selection for postweaning gain in mice. Genetics, Princeton 73, 513-530.

HANRAHAN, J.P., HOOPER, A.C. & McCARTHY, J.C. (1973b) Effects of divergent selection for body weight on fibre number and diameter in two mouse muscles. Anim. Prod. 16, 7-16.

HARINGTON, C.R. & BARGER, G. (1927) Chemistry of thyroxine. III Constitution and synthesis of thyroxine. Biochem. J. 21, 169-181.

HARRIS, C.I., RUCKLIDGE, G.J., McDIARMID, R.M. & MILNE, G. (1986) Sex- and phenotype-dependent metabolism of N^t-methylhistidine. Biochem. J. 239, 229-232.

HARVEY, W.R. (1972) Direct and indirect responses in two-trait selection experiments in mice. Proc. 21st Annual Poultry Breeders' Roundtbable, Poultry Breeders of America, Kansas City, Mo., pp71-95.

HASSELGREN, P-O., ADLERBERTH, A., ANGERAS, U. & STENSTROM, G. (1984) Protein metabolism in skeletal muscle tissue from hyperthyroid patients after preoperative treatment with antithyroid drug or selective β-blocking agent: Results from a prospective randomized study. J. clin. Endocr. Metab. 59(5), 835-839.

HAYASHI, K., KAYALI, A.G. & YOUNG, V.R. (1986) Synergism of triiodothyronine and cortisone on muscle protein breakdown. Biochim. biophys. Acta 883, 106-111.

HAYES, J.F. & McCARTHY, J.C. (1976) The effects of selection at different ages for high and low body weight on the pattern of fat deposition in mice. Genet. Res. 27, 389-403.

HEFCO, E., KRULICH, L., ILLNER, P. & LARSEN, P.R. (1975) Effect of acute exposure to cold on the activity of the hypothalamic-pituitary-thyroid system. Endocrinology 97, 1185-1195.

HEITZMAN, R.J. & CHAN, K.H. (1974) Alterations in weight gain and levels of plasma metabolites, proteins, insulin and free fatty acids following implantation of an anabolic steroid in heifers. Br. vet. J. 130, 532-537.

HEITZMAN, R.J., CHAN, K.H. & HART, I.C. (1977) Liveweight gains, blood levels of metabolites, proteins and hormones following implantation of anabolic agents in steers. Br. vet. J. 133, 62-70.

HERSKO, A. & CIECHANOVER, A. (1982) Mechanisms of intracellular protein breakdown. A. Rev. Biochem. 51, 335-364.

HERSKO, A., CIECHANOVER, A., HELLER, H., HAAS, A.L. & ROSE, I.A.

(1980) Proposed role of ATP in protein breakdown: conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis. Proc. natn. Acad. Sci. USA. 77, 1783-1786.

HETZEL, D.J.S. & NICHOLAS, F.W. (1978) Growth and body composition of mice selected for growth rate under ad libitum or restricted feeding. Proc. Aust. Soc. Anim. Prod. 12, 194.

HILL, J.M. & MALAMUD, M. (1974) Decreased protein catabolism during stimulated growth. FEBS Lett. 46, 308-311.

HILLGARTNER, F.B. & ROMSOS, D.R. (1985) Regulation of iodothyronine 5'-deiodination in lean and obese (ob/ob) mice. Am. J. Phsiol. 249, E209-E218.

HILLIER, A.P. (1972) Deiodination of thyroid hormones by the perfused rat liver. J. Physiol., Lond. 222, 475-485.

HIMMS-HAGEN, J. (1976) Cellular thermogenesis. A. Rev. Physiol. 38, 315-351.

HOLDER, A.T. & WALLIS, M. (1976) Regulation of serum somatomedin levels and growth in dwarf mice by growth hormone, prolactin and thyroxine. J. Endocr. 71, 82P.

HOLDER, A.T. & WALLIS, M. (1977) Actions of growth hormone, prolactin and thyroxine on serum somatomedin-like activity and growth in hypopituitary dwarf mice. J. Endocr. 74, 223-229.

HOLDSWORTH, C.D. & BESSER, H.M. (1968) Influence of gastric emptying-rate and of insulin response on oral glucose tolerance in thyroid disease. Lancet i, 700-702.

HOOPER, A.C.B. (1976) Longitudinal growth of skeletal muscle fibres in lines of mice selected for high and low body weight. Growth 40, 33-39.

HOOPER, A.C.B. & HURLEY, M.P. (1983) The effect of selection for altered body weight on the ultrastructure components of skeletal muscle fibres. Anim. Prod. 36, 223-227.

HOOPER, A.C.B. & McCARTHY, J.C. (1976) A note on fibre number and diameter in muscles of large and small lines of mice compared at a fixed body weight. Anim. Prod. 22, 131-133.

HUANG, M-T. & LARDY, H.A. (1981) Effects of thyroid status on

the Cori cycle, glucose-alanine cycle, and futile cycling of glucose metabolism in rats. Archs. Biochem. Biophys. 209(1), 41-51.

HULL, P. (1960) Genetic relations between carcass fat and body weight in mice. J. agric. Sci., Camb. 55, 317-321.

HURST, V. & TURNER, C.W. (1948) The thyroid secretion rate in the mouse and its relation to various physiological processes. Univ. Missouri Agr. Exp. Stn. Res. Bull. 417, 1-62.

IMURA, H., TANAKA, K., MASHIO, Y., ISHII, H., INADA, M. & NISHIKAWA, M. (1984) In Hormone Receptors in Growth and Reproduction (Saxena, B.B., Catt, K.J., Birnbaumer, L. and Matini, L., eds.) pp331-340, Raven Press, New York.

INGENBLEEK, Y. & BECKERS, C. (1975) Triiodothyronine and thyroid-stimulating hormone in protein-calorie malnutrition in infants. Lancet ii, 845-848.

ISAKSSON, O.G.P., EDEN, S. & JANSSON, J-O. (1985) Mode of action of pituitary growth hormone on target cells. A. Rev. Physiol. 47, 483-499.

JACOBS, B.B. (1958) Variations in thyroid morphology of mice. Proc. Soc. exp. Biol. Med. 97, 115-118.

JEFFERSON, L.S., RANNELS, D.E., MUNGER, B.L. & MORGAN, H.E. (1974) Insulin in the regulation of protein turnover in heart and skeletal muscle. Fedn. Proc. 33, 1098-1104.

JUNG, R.T., SHETTY, P.S., BARRAND, M., CALLINGHAM, B.A. & JAMES, W.P.T. (1978) The role of catecholamines and thyroid hormones in the metabolic response to semistarvation. 38, 17A.

KARSTEN, U. & WOLLENBERGER, A. (1972) Determination of DNA and RNA in homogenized cells and tissues by surface fluorometry. Analyt. Biochem. 46, 135-148.

KASSER, T.R., MARTIN, R.J., GAHAGAN, J.H. & WANGSNESS, P.J. (1981) Fasting plasma hormones and metabolites in feral and domestic newborn pigs. J. Anim. Sci. 53, 420-425.

KELLY, F.J. & GOLDSPINK, D.F. (1982) The differing responses of four muscle types to dexamethasone treatment in the rat. Biochem. J. 208, 147-151.

KEMPSTER, A.J. & CUTHBERTSON, A. (1977) A survey of the carcass characteristics of the main types of British lamb. Anim. Prod. 25, 165-179.

KEMPSTER, A.J., CUTHBERTSON, A. & HARRINGTON, G. (1976) Fat distribution in steer carcasses of different breeds and crosses. Anim. Prod. 23, 25-34.

KIELANOWSKI, J. (1968) The method of pig progeny testing applied in Poland. I General principles and physiological background. Proc. meeting of Sub-Commission on Progeny Testing. 9th Study Meeting of EAAP, Dublin.

KING, D.B. & KING, C.R. (1973) Thyroidal influence on early muscle growth of chickens. Gen. comp. Endocr. 21, 517-529.

KLEIBER, M. (1961) in The Fire of Life, Wiley, New York.

KONITS, P.H., HAMILTON, B.P., PRUCE, E.S., WHITACRE, M. & Van ECHO, D.H. (1984) Serum thyroid changes during whole body hyperthermia. Cancer, N.Y. 54(11), 2432-2435.

KOWNACKI, M. & JEZIERSKI, T. (1980) Effect of selection on some physiological and biochemical traits in mice. 31st Annual Meeting, European Assocn. for Animal Procuction No G1.12, p7.

KOWNACKI, M. & KELLER, J. (1978) The basal metabolic rate in selected and unselected mice. Genet. Polon. 19(3), 339-343.

KOWNACKI, M., KELLER, J. & GEBLER, E. (1975) Selection of mice for high weight gains - its effect on the basal metabolic rate. Genet. Polon. 16(3-4), 359-363.

KRUPP, P.P., STARLING, J.R., GOLSTEIN, J. & NEVE, P. (1984) Thyroid lysosomal enzyme activity and ultrastructure after subtotal thyroidectomy. Endocrinology 115(5), 1905-1912.

KUHN, E.R., BELLON, K., HUYBRECHTS, L. & HEYNS, W. (1983) Endocrine differences between the Wistar and Sprague-Dawley laboratory rat: influence of cold adaption. Horm. metab. Res. 15, 491-498.

KVETNY, J. (1985) Nuclear thyroxine receptors and cellular metabolism of thyroxine in obese subjects before and after fasting. Horm. Res., Basel 21(1), 60-65.

LAND, R.B. (1981) Physiological criteria and genetic selection. Livest. Prod. Sci. 8, 203-213.

LANG, B.J. & LEGATES, J.E. (1969) Rate, composition and efficiency of growth in mice selected for large and small body weight. Theor. Appl. Genet. 39, 306-314.

LARSEN, P.R., SILVA, J.E. & KAPLAN, M.M. (1981) Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications. Endocr. Rev. 2(1), 87-102.

LAURENT, G.J., SPARROW, M.P. & MILLWARD, D.J. (1978) Turnover of muscle protein in fowl. Changes in rates of protein synthesis and breakdown during hypertrophy of the anterior and posterior latissimus dorsi muscles. Biochem. J. 176, 407-417.

LERNER, I.M. (1954) in Genetic Homeostasis , Oliver and Boyd, Edinburgh.

LEWIS, S.E.M., KELLY, F.J. & GOLDSPINK, D.F. (1984) Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. Biochem. J. 217, 517-526.

LI, J.B & GOLDBERG, A.L. (1976) Effects of food deprivation on protein synthesis and degradation in rat skeletal muscles. Am. J. Physiol. 231(1), 441-448.

LIN, M.H., VANDER TUIG, J.G., ROMSOS, D.R., AKERA, T. & LEVEILLE, G.A. (1979) Na^+,K^+-ATPase enzyme units in lean and obese (ob/ob) thyroxine-injected mice. Am. J. Physiol. 237, E265-E272.

LOBLEY, G.E., REEDS, P.J. & PENNIE, K. (1978) Protein synthesis in cattle. Proc. Nutr. Soc. 37, 96A.

LOEB, J.N. (1979) in The Thyroid (Ingbar, S.H. & Werner, S.C., eds.), pp705-712 & pp872-877, Harper and Row, New York.

LONDON, I.M., CLEMENS, M.J., RAMU, R.S., LEVIN, D.H., CHERBAS, L.F. & ERNST, V. (1976) The role of hemin in the regulation of protein synthesis in erythroid cells. Fedn. Proc. 35, 2218-2222.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin Phenol Reagent. J. biol. Chem. 193, 265-275.

LUFF, A.R. & GOLDSPINK, G. (1970) Total number of fibres in muscles of several strains of mice. J. Anim. Sci. 30, 891-893.

MacARTHUR, J.W. (1944) Genetics of body size and related characters II Satellite characters associated with body size in mice. Am. Nat. 78, 224-237.

McCARTHY, J.C. (1971) Effects of different methods of selection for weight on the growth curve in mice. Xth International Conference on Animal Production.

McCARTHY, J.C. (1979) in Animal Models of Obesity (Festing, M.F.W., ed.), pp1-14, The MacMillan Press Ltd.

McCARTHY, J.C. (1982) The laboratory mouse as a model for animal breeding: a review of selection for increased body weight and litter size. Proc. 2nd World Congress on Genetics Applied to Livestock Production, Madrid. 5, 66-83.

McCARTHY, J.C. & BAKKER, H. (1979) The effects of selection for different combinations of weights at two ages on the growth curve of mice. Theor. Appl. Genet. 55, 57-64.

McCARTHY, J.C., PETERSON, S., GRAHAM, A. & WILLEKE, H. (1978-79) Efficiency of food utilisation in large and small lines of mice. Irish Republic, Univ. College, Dublin, Faculty of General Agriculture. Research report 1978-79, Dublin, Irish Republic.

McELLIGOTT, M.A. & DICE, J.F. (1983) Erythrocyte-mediated microinjection, a technique to study protein degradation in muscle cells. Biochem. J. 216, 559-566.

McGRATH, J.A. & GOLDSPINK, D.F. (1978) The effects of cortisone treatment on the protein turnover of the soleus muscle after immobilization. Biochem. Soc. Trans. 6, 1017-1019.

McGRATH, J.A. & GOLDSPINK, D.F. (1982) Glucocorticoid action on protein synthesis and protein breakdown in isolated skeletal muscles. Biochem. J. 206, 641-645.

McLELLAN, C.R.,Jr., & FRAHM, R.R. (1973) Direct and correlated responses to two-way selection for hindleg muscle system weight in mice. J. Anim. Sci. 36, 442-451.

McNURLAN, M.A. & GARLICK, P.J. (1979) Rates of protein synthesis in rat liver and small intestine in protein deprivation and diabetes. Proc. Nutr. Soc. 38(3), 133A.

McNURLAN, M.A., TOMKINS, A.M. & GARLICK, P.J. (1979) The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem. J. 178, 373-379.

McNURLAN, M.A., PAIN, V.M. & GARLICK, P.J. (1980) Conditions that alter rates of tissue protein synthesis in vivo. Biochem. Soc. Trans. 8(3), 283-285.

McPHEE, C.P. & TRAPPETT, P.C. (1987) Growth and body composition changes in mice selected for high post-weaning weight gain on two levels of feeding. Theor. Appl. Genet. 73, 926-931.

McPHEE, C.P., TRAPPETT, P.C., NEILL, A.R. & DUNCALFE, F. (1980) Changes in growth, appetite, food conversion efficiency and body composition in mice selected for high post-weaning weight gain on restricted feeding. Theor. Appl. Genet. 57, 49-56.

MARECHAUD, R., RAMBAUD, J.C. & MARTUCHANSKY, C. (1980) Intestinal manifestations of thyroid disease: current clinical and pathological aspects. Gastroenterol. Clin. Biol. 4, 899-910.

MARTIN, A.H., FREEDEN, H.T., WEISS, G.M. & CARSON, R.B. (1972) Distribution and composition of porcine carcass fat. J. Anim. Sci. 35, 534-541.

MARTINEZ, J.A., BUTTERY, P.J. & PEARSON, J.T. (1984) The mode of action of anabolic agents: the effect of testosterone on muscle protein metabolism in the female rat. Br. J. Nutr. 52, 515-521.

MARTYNENKO, F.P. & KORNYUSHENKO, N.P. (1984) Somatotropin influence on cathepsin D activity in the liver of hypothyroid and hyperthyroid rats. Probl. Endokr. 30(3), 60-64.

MARUYAMA, K., SUNDE, M.L. & SWICK, R.W. (1978) Growth and muscle protein turnover in the chick. Biochem. J. 176, 573-582.

MATHEWS, R.W., ORONSKY, A. & HASCHEMEYER, A.E.V. (1973) Effect of thyroid hormone on polypeptide chain assembly kinetics in liver protein synthesis in vivo. J. biol. Chem. 248(4), 1329-1333.

MAYER, M. & ROSEN, F. (1975) Interaction of anabolic steroids with glucocorticoid receptor sites in rat muscle cytosol. Am. J. Physiol 229(5), E1381-E1386.

MAYER, R.J. (1978) Enzyme turnover in differentiating and developing cells. Biochem. Soc. Trans. 6, 505-509.

MAYER, R.J. & DOHERTY, F. (1986) Intracellular protein catabolism: state of the art. FEBS Rev. Lett. 198(2), 181-193.

MEYER, H.H. & BRADFORD, G.E. (1974) Estrus, ovulation rate and body composition of selected strains of mice on ad libitum and restricted feed intake. J. Anim. Sci. 38(2), 271-278.

MILLWARD, D.J. (1970) Protein turnover in skeletal muscle. II The effect of starvation and a protein-free diet on the synthesis and catabolism of skeletal muscle proteins in comparison to liver. Clin. Sci. 39, 591-603.

MILLWARD, D.J. (1978) The regulation of muscle-protein turnover during growth and development. Biochem. Soc. Trans. 6, 494-499.

MILLWARD, D.J. & LO, C.C. (1977) The effect of fasting and refeeding on whole-body protein synthesis in the rat. Proc. Nutr. Soc. 36, 137A.

MILLWARD, D.J. & WATERLOW, J.C. (1978) Effect of nutrition on protein turnover in skeletal muscle. Fedn. Proc. Fedn.Am. Socs. exp. Biol. 37, 2283.

MILLWARD, D.J., GARLICK, P.J., JAMES, W.P.T., NNANYELUGO, D.O. & RYATT, J.S. (1973) Relationship between protein synthesis and RNA content of skeletal muscle. Nature, Lond. 241, 204-205.

MILLWARD, D.J., NNANYELUGO, D.O. & GARLICK, P.J. (1974a) Developmental changes in muscle protein metabolism in congenitally malnourished rats. Proc. Nutr. Soc. 33, 55A.

MILLWARD, D.J., NNANYELUGO, D.O. & GARLICK, P.J. (1974b) The effect on muscle protein metabolism of refeeding protein-depleted rats. Proc. Nutr. Soc. 33, 115A.

MILLWARD, D.J., GARLICK, P.J., STEWART, R.J.C., NNANYELUGO, D.O. & WATERLOW, J.C. (1975) Skeletal-muscle growth and protein turnover. Biochem. J. 150, 235-243.

MILLWARD, D.J., GARLICK, P.J., NNANYELUGO, D.O. & WATERLOW, J.C. (1976) The relative importance of muscle protein synthesis and breakdown in regulation of muscle mass. Biochem. J. 156, 185-188.

MILLWARD, D.J., HOLLIDAY, M.A., BATES, P.C., DALAL, S., COX, M. & HEARD, C.R.C. (1979) The relationship between dietary state, thyroid hormones, oxygen consumption and muscle protein turnover. Proc. Nutr. Soc. 38, 33A.

MILLWARD, D.J., ODEDRA, B. & BATES, P.C. (1983) The role of insulin, corticosterone and other factors in the acute recovery of muscle protein synthesis on refeeding food-deprived rats. Biochem. J. 216, 583-587.

MORE O'FERRALL, G.J. & TIMON, V.M. (1970) Direct selection for tissue weight and correlated responses in lines selected for weight in mice. Br. Soc. Anim. Prod., Harrogate.

MORTIMORE, G.E. & WARD, W.F. (1981) Internalization of cytoplasmic protein by hepatic lysosomes in basal and deprivation-induced proteolytic states. J. biol. Chem. 256, 7659-7665.

MORTIMORE, G.E., NEELY, A.N., COX, J.R. & GUINIVAN, R.A. (1973) Proteolysis in homogenates of perfused rat liver: responses to insulin, glucagon and amino acids. Biochem. biophys. Res. Commun. 54, 89-95.

MUIR, L.A., WIEN, S., DUQUETTE, P.F., RICKES, E.L. & CORDES, E.H. (1975) Effects of exogenous growth hormone and diethylstilbesterol on growth and carcass composition of growing lambs. J. Anim. Sci. 56, 1315-1323.

MULLER, M.J. & SEITZ, H.J. (1980) Rapid and direct stimulation of hepatic gluconeogenesis by L-triiodothyronine in the isolated perfused rat liver. Life Sci. 27, 827-835.

MULLER, M.J., PASCHEN, U. & SEITZ, H.J. (1983) Thyroid hormone regulation of glucose homeostasis in the miniture pig. Endocrinology 112, 2025-2031.

MULVANEY, D.R., MERKEL, R.A. & BERGEN, W.G. (1985) Skeletal muscle protein turnover in young male pigs. J. Nutr. 115, 1057-1064.

MURAKAMI, T., HATANAKA, M. & MURACHI, T. (1981) The cytosol of human erythrocytes contains a highly Ca^{2+}-sensitive thiol protease (calpain I) and its specific inhibitor protein (calpastatin). J. Biochem., Tokyo 90, 1809-1816.

MURAMATSU, T., AOYAGI, Y., OKUMURA, J. & TASAKI, I. (1987) Contribution of whole-body protein synthesis to basal metabolism in layer and broiler chickens. Br. J. Nutr. 57, 269-277.

MURRAY, A.J., NEILD, M.K., JONES, L.M., GALBRAITH, N. & TOMAS, F.M. (1985) Metabolism of N^{τ}-methylhistidine by mice. Biochem. J. 232, 409-413.

NEELY, A.N., NELSON, P.B. & MORTIMORE, G.E. (1974) Osmotic alterations of the lysosomal system during rat liver perfusion: reversible suppression by insulin and amino acids. Biochim. biophys Acta 338, 458-472.

OKAJIMA, F. & UI, M. (1979) Metabolism of glucose in hypo- and hyperthyroid rats in vivo. Biochem. J. 182, 565-575.

OPPENHEIMER, J.H., SCHWARTZ, H.L., SURKS, M.I., KOERNER, D. & DILLMAN, W.H. (1976) Nuclear receptors and the initiation of thyroid hormone action. Rec. Prog. Horm. Res. 32, 529-565.

ORTIZ-CARO, J., GONZALEZ, C. & JOLIN, T. (1984) Diurnal variations of plasma growth hormone, thyrotropin, thyroxine and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115(6), 2227-2232.

PAIN, V.M. & CLEMENS, M.J. (1980) in Comprehensive Biochemistry Vol. 19B part 1 (Florkin, M., Neuberger, A. & Van Deenan, L.L.M., eds.), pp1-76, Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York.

PAIN, V.M., ALBERTSE, E.C. & GARLICK, P.J. (1983) Protein metabolism in skeletal muscle, diaphragm and heart of diabetic rats. Am. J. Physiol. 245, E604-E610.

PALMER, L.S., KENNEDY, C., CALVERLEY, C.E., LOHN, C. & WESTWIG, P.H. (1946) Genetic differences in the biochemistry and physiology influencing food utilization for growth in rats. Tech. Bull. No. 176, Univ. Minnesota Agric. Exp. Stn.

PALMER, R.M., BAIN, P.A. & REEDS, P.J. (1985) The effect of insulin and intermittent mechanical stretching on rates of protein synthesis and degradation in isolated rabbit muscle. Biochem. J. 230, 117-123.

PEAVY, D.E., TAYLOR, J.M. & JEFFERSON, L.S. (1981) Protein synthesis in perfused rat liver following thyroidectomy and hormone treatment. Am. J. Physiol. 240, E18-E23.

PELL, J.M., BATES, P.C., McANULTY, R.J. & LAURENT, G.J. (1988) The use of [^{3}H] proline to determine rates of collagen and noncollagen protein synthesis in skeletal muscle of lambs: effects of growth hormone. Proc. Nutr. Soc. in press.

PELL, J.M., BLAKE, L.A., BUTTLE, H.L., JOHNSSON, I.D. & SIMMONDS, A.D. (1987) Insulin-like growth factor-I and growth hormone in sheep. Proc. Nutr. Soc. 46, 48A.

PETERSEN, S. & McCARTHY, J.C. (1981) Feeding behaviour of large and small lines. Irish Republic, Univ. College, Dublin, Faculty of General Agriculture. Research report 1978-79, Dublin, Irish Republic p185.

PIDDUCK, H.G. & FALCONER, D.S. (1978) Growth hormone function in strains of mice selected for large and small size. Genet. Res. 32, 195-206.

POMEROY, R.W. & WILLIAMS, D.R. (1974) The partition of fat in the bovine carcass. Proc. Br. Soc. Anim. Prod. 3, 85A.

PREEDY, V.R., McNURLAN, M.A. & GARLICK, P.J. (1983) Protein synthesis in skin and bone of the young rat. Br. J. Nutr. 49, 517-523.

PRESTON, R.L. (1975) Biological responses to estrogen additives in meat producing cattle and lambs. J. Anim. Sci. 41, 1414.

PRIESTLEY, G.C. & ROBERTSON, M.S.M. (1973) Protein and nucleic acid metabolism in organs from mice selected for larger and smaller body size. Genet. Res. 22, 255-278.

PUERIA, M.L., ABELENDA, M. & FRAILE, A. (1984) Effects of thyroxine and 3,5,3'-triiodothyronine in brown adipose tissue of rats [Rattus novegicus]. Comp. Biochem. Physiol. A. Comp. Physiol. 79(4), 563-566.

PULLAR, J.D. & WEBSTER, A.J.F. (1974) Heat loss and energy retention during growth in congenitally obese and lean rats. Br. J. Nutr. 31, 377-392.

PULLAR, J.D. & WEBSTER, A.J.F. (1977) The energy cost of fat and protein deposition in the rat. Br. J. Nutr. 37, 355-363.

RAAKA, B.M. & SAMUELS, H.H. (1981) Regulation of thyroid hormone nuclear receptor levels in GH cells by 3,5,3'-triiodo-L-thyronine. J. biol. Chem. 256, 6883-6889.

RABINOVITZ, M. & FISHER, J.M. (1962) Formation of a ribosomal lesion in rabbit reticulocytes by the lysine antagonist, S-(β-aminoethyl) cysteine. Biochem. biophys. Res. Commun. 6, 449-451.

RABINOVITZ, M. & FISHER, J.M. (1964) Characteristics of the inhibition of hemoglobin synthesis in rabbit reticulocytes by threo-α-amino-β-chlorbutyric acid. Biochim. biophys. Acta 91,

313-322.

RADCLIFFE, J.D. & WEBSTER, A.J.F. (1976) Regulation of food intake during growth in fatty and lean female Zucker rats given diets of different protein content. Br. J. Nutr. 36, 457-469.

RAHNEFELD, G.W., BOYLAN, W.J., COMSTOCK, R.E. & SINGH, M. (1963) Mass selection for post-weaning growth in mice. Genetics, Princeton 48, 1567-1583.

RAHNEFELD, G.W., COMSTOCK, R.E., BOYLAN, W.J. & SINGH, M. (1965) Genetic correlation between growth rate and feed per unit gain in mice. J. Anim. Sci. 24, 1061-1066.

REEDS, P.J. & PALMER, R.M. (1983) The possible involvement of prostaglandin $F_{2\alpha}$ in the stimulation of muscle protein synthesis by insulin. Biochem. biophys. Res. Commun. 116(3), 1084-1090.

RICHMOND, R.J. & BERG, R.T. (1971) Tissue development in swine as influenced by live-weight, breed, sex and ration. Can. J. Anim. Sci. 51, 31-39.

RIKIMARU, T., YAMAMOTO, S., MAEDA, K. & INOUE, G. (1980) Effects of protein deficiency on muscle myofibrillar protein turnover in adult rats. J. Nutr. Sci. Vitaminol. 26, 39-57.

RIOS, J.G., NIELSEN, M.K., DICKERSON, G.E. & DeSHAZER, J.A. (1986) Selection for postweaning gain in rats: I Correlated response in feed utilization and body composition. J. Anim. Sci. 63, 34-45.

ROBERTS, R.C. (1961) The lifetime growth and reproduction of selected strains of mice. Heredity, Lond. 16, 369-381.

ROBERTS, R.C. (1974) Food consumption in mice selected for high and low body weight. Genetics, Princeton 74, 2.2A.

ROBERTS, R.C. (1981) The growth of mice selected for large and small size in relation to food intake and the efficiency of conversion. Genet. Res. 38, 9-24.

ROBINSON, D.W. & BRADFORD, G.E. (1969) Cellular response to selection for rapid growth in mice. Growth 33, 221-229.

ROCHE, J., LISSITZKY, S. & MICHEL, R.C. (1952) Sur la presence de triiodothyronine dans la thyroglobuline. C.r. hebd. Seance.

Acad. Sci., Paris. 234, 1228-1230.

ROGNSTAD, R. (1977) Futile hydrogen cycling in liver cells from triiodthyronine treated rats. Biochem. biophys. Res. Commun. 78, 881-888.

ROMSOS, D.R. & LEVEILLE, G.A. (1974) Body fat and fatty acid synthesis in five lines of mice selected for growth rate. Proc.Soc. exp. Biol. Med. 145, 591-594.

RUPP, J., PASCHLEIS, K.E. & CANTAROW, A. (1949) Influence of thyroxine on protein metabolism. Endocrinology 44, 449-453.

RYG, M. (1984) Seasonal changes in the relationship between food intake and serum triidothyronine in reindeer [Rangifer tarandus tarandus]. Comp. Biochem. Physiol. A. Comp. Physiol. 78(3), 427-430.

SAMUELS, H.H., PERLMAN, A.J., RAAKA, B.M. & STANLEY, F. (1982) Organization of the thyroid hormone receptor in chromatin. Rec. Prog. Horm. Res. 38, 557-599.

SANFORD, C.F., GRIFFIN, E.E. & WILDENTHAL, K. (1978) Synthesis and degradation of myocardial protein during the development and regression of thyroxine-induced cardiac hypertrophy in rats. Circulation Res. 43, 688-694.

SAUNDERSON, C.L. & BRYAN, L. (1985) Comparison of muscle protein degradation rates in two strains of young chicks by using N^{τ}-methylhistidine excretion. Biochem. Soc. Trans. 13, 342.

SCHWEIGER, H.G., RAPOPORT, S. & SCHOLZEL, E. (1956) Role of non-protein nitrogen in the synthesis of haemoglobin in the reticulocyte in vitro. Nature, Lond. 178. 141-142.

SCOW, R.O. (1954) Effect of thyroxine on the weight and composition of muscle, pelt and other tissues in young hypophysectomized rats. Endocrinology 55, 344-353.

SEEBECK, R.M. (1968) A dissection study of the distribution of tissues in lamb carcasses. Proc. Aust. Soc. Anim. Prod. 7, 297-302.

SESTOFT, L. (1980) Metabolic aspects of the calorigenic action of thyroid hormones in mammals. Clin. Endocr. 13, 489-506.

SHARP, G.L., HILL, W.G. & ROBERTSON, A. (1984) Effects of selection on growth, body composition and food intake in mice. I Responses in selected traits. Genet. Res. 43, 75-92.

SILVERSTEIN, E., SOKOLOFF, L., MICKELSEN, O. & JAY, G.E.,Jr., (1960) Thyroid function in various strains of mice: T/S ratio, PBI and thyroid weight. Am. J. Physiol. 199, E203-E208.

SINNETT-SMITH, P.A., PALMER, C.A. & BUTTERY, P.J. (1987) Androgen receptors in skeletal muscle cytosol from sheep treated with trenbolone acetate. Horm. metab. Res. 19, 110-114.

SMITH, T.J. & EDELMAN, I.S. (1979) The role of sodium transport in thyroid thermogenesis. Fedn. Proc. 38, 2150-2153.

SORENSEN, M.T., KRUSE, V. & ANDERSEN, B.B. (1981) Thyroxine degradation in young bulls of Danish dual-purpose breeds. Genetic relationship to weight gain, feed conversion and breeding value for butterfat production. Livest. Prod. Sci. 8, 399-406.

SPAULDING, S.W., CHOPRA, I.J., SHERWIN, R.S. & LYALL, S.S. (1976) Effect of caloric restriction and dietary composition on serum T_3 and reverse T_3 in man. J. clin. Endocr. Metab. 42, 197-200.

SPENCER, G.S.G. & WILLIAMSON, E.D. (1981) Increased growth in lambs following immunisation against somatostatin. Anim. Prod. 32, 69A.

SPENCER, G.S.G., GARSSEN, G.J. & HART, I.C. (1983) A novel approach to growth promotion using autoimmunisation against somatostatin. I Effects on growth and hormone levels in lambs. Livest. Prod. Sci. 10, 25-37.

STANIER, M.W. & MOUNT, L.E. (1972) Growth rate, food intake and body composition before and after weaning in strains of mice selected for mature body-weight. Br. J. Nutr. 28, 307-325.

STERLING, K. (1979) Thyroid hormone action at cell level. N. Engl. J. Med. 300, 117-123 & 173-177.

STEWART, P.A. & WASHBURN, K.W. (1983) Variation in growth hormone, triiodothyronine (T_3) and lipogenic enzyme activity in broiler strains differing in growth and fatness. Growth 47, 411-425.

SUTHERLAND, T.M., BIONDINI, P.E., HAVERLAND, L.H., PETTUS, D. &

OWEN, W.B. (1970) Selection for rate of gain, appetite and efficiency of feed utilization in mice. J. Anim. Sci. 31, 1049-1057.

SUTHERLAND, T.M., BIONDINI, P.E. & WARD, G.M. (1974) Selection for growth rate, feed efficiency and body composition in mice. Genetics, Princeton 78, 525-540.

SUZUKI, O. & YAGI, K. (1976) A fluorometric assay for $-phenylethylamine in rat brain. Analyt. Biochem. 75, 192-200.

SYNENKI, R.M., EISEN, E.J., MATRONE, G. & ROBISON, O.W. (1972) Thyroid activity in lines of mice selected for large and small size. Can. J. Genet. Cytol. 14, 483-494.

TAYLOR, St.C.S. (1973) Genetic differences in milk production in relation to mature body weight. Proc. Br. Soc. Anim. Prod. 2, 15-26.

TAYLOR, St.C.S. (1980a) Genetic size-scaling rules in animal growth. Anim. Prod. 30, 161-165.

TAYLOR, St.C.S. (1980b) Genetically standardized growth equations. Anim. Prod. 30, 167-175.

THOMAS, K.M. & RODWAY, R.G. (1982) Suppression of adrenocortical function in rats and sheep with the anabolic steroid trenbolone acetate. Proc. Nutr. Soc. 41, 138A.

THOMPSON, E.B. & LIPPMAN, M.E. (1974) Mechanism of action of glucocorticoids. Metabolism 23(2), 159-202.

TILAKARATNE, N., HILL, W.G. & LAND, R.B. (1981) Direct and correlated responses to selection for plasma thyroxine levels in mice. Genet. Res. 38, 157-169.

TIMON, V.M. & EISEN, E.J. (1969) Comparison of growth curves of mice selected and unselected for postweaning gain. Theor. Appl. Genet. 39, 345-351.

TIMON, V.M. & EISEN, E.J. (1970) Comparison of ad libitum and restricted feeding of mice selected and unselected for postweaning gain. I Growth, feed consumption and feed efficiency. Genetics, Princeton 64, 41-57.

TIMON, V.M., EISEN, E.J. & LEATHERWOOD, J.M. (1970) Comparisons

of ad libitum and restricted feeding of mice selected for postweaning gain. II Carcass composition and energetic efficiency. Genetics, Princeton 65, 145-155.

TRENKLE, A. (1976) in Anabolic Agents in Animal Production (Lu, F.C. & Rendel, J., eds.), pp79-88, Georg Thieme Publications, Stuttgart.

VAN BUUL-OFFERS, S., SMEETS, T. & VAN den BRANDE J.L. (1984) Effects of growth hormone and thyroxine on the relation between tibial length and the histological appearance of the proximal tibial epiphysis in Snell dwarf mice. Growth 48(2), 166-175.

VERNON, B.G. & BUTTERY, P.J. (1976) Protein turnover in rats treated with trienbolone acetate. Br. J. Nutr. 36, 575-579.

WANG, C-S. & SMITH, R.L. (1975) Lowry determination of protein in the presence of Triton-X-100. Analyt. Biochem. 63, 414-417.

WEBSTER, A.J.F. (1977) Selection for leanness and the energetic efficiency of growth in meat animals. Proc. Nutr. Soc. 36, 53-59.

WEBSTER, A.J.F. (1980) The energetic efficiency of growth. Livest. Prod. Sci. 7, 243-252.

WEBSTER, A.J.F. (1981) The energetic efficiency of metabolism. Proc. Nutr. Soc. 40, 121-127.

WEBSTER, A.J.F., LOBLEY, G.E., REEDS. P.J. & PULLAR, J.D. (1978) Protein mass, protein synthesis and heat loss in the Zucker rat. Proc. Nutr. Soc. 37, 21A.

WEBSTER, A.J.F., LOBLEY, G.E., REEDS, P.J. & PULLAR, J.D. (1980) in Energy Metabolism, Proc. 8th Symposium on Energy Metabolism (Mount, L.E., ed.), pp125-128.

WILKINSON, K.D., URBAN, M.K. & HAAS, A.L. (1980) Ubiquitin is the ATP-dependent proteolysis factor I of rabbit reticulocytes. J. biol. Chem. 255, 7529-7532.

WILSON, J.W. (1952) Response of various inbred mouse strains to the goitrogenic action of sulfadiazine. Anat. Rec. 113, 86A.

WILSON, S.P. (1973) Selection for a ratio of body weight gain in mice. J. Anim. Sci. 37(5), 1098-1103.

WILSON, S.P., GOODALE, H.D., KYLE, W.H. & GODFREY, E.F. (1971) Long term selection for body weight in mice. J. Hered. 62, 228-234.

WOOD, D.F., FRANKLYN, J.A., DOCHERTY, K., RAMSDEN, D.B. & SHEPPARD, M.C. (1987) The effect of thyroid hormones on growth hormone gene expression in vivo in rats. J. Endocr. 112, 459-463.

WYKES, A.A., CHRISTIAN, J.E. & ANDREWS, F.N. (1958) Radioiodine concentration and thyroid weight in normal, obese and dwarf strains of mice. Endocrinology 62, 535-538.

YOUNG, V.R. (1980) in Protein Deposition in Animals, 29th Easter School, Nottingham University, (Buttery, P.J. & Lindsay, D.B., eds.), pp167-191, Butteworths, London.

ZEMAN, R.J., BERNSTEIN, P.L., LUDEMANN, R. & ETLINGER, J.D. (1986) Regulation of Ca^{2+}-dependent protein turnover in skeletal muscle by thyroxine. Biochem. J. 240, 269-272.